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DISSERTATION

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Chapter 1

General introduction

In 1992, a report from the Adventist Health Study was published which suggested that frequent nut consumption might protect against coronary heart disease (1). Shortly thereafter, a diet enriched with walnuts was shown to improve serum lipid levels in a clinical trial (2). Subsequent publications on nut intake and cardiovascular diseases eventually led to a qualified health claim, published by the US Food and Drug Administration in 2003, on the relation between nut consumption and a reduced risk of coronary heart disease and intermediate biomarkers, like blood cholesterol (3). Since then, the interest in the health effects of nut intake has been growing rapidly.

Overview of nut and peanut butter consumption

When referring to nuts, we can distinguish between tree nuts and peanuts. Tree nuts are defined as dry fruits with one seed, in which the ovary wall becomes hard and though during maturation (4, 5). Common edible tree nuts include almonds, hazelnuts, walnuts, pine nuts, pistachios, cashews, macadamia nuts, and pecans. Frequently, peanuts are also considered as nuts, while they are botanically groundnuts or legumes (4). The nutrient profile of peanuts is quite similar to that of tree nuts: both are nutrient dense and contain high levels of mono- and polyunsaturated fatty acids, vegetable protein, dietary fiber, vitamins (e.g. niacin, B6, folate, tocopherols), minerals (e.g. magnesium, calcium, potassium, selenium), phytosterols and phenolic compounds (4, 6). Many of these nutrients have been associated with a reduced risk of several chronic diseases (4, 7). In contrast, chestnuts are tree nuts by definition, but are more starchy and contain little fat, and thus have a completely different nutrient profile than other tree nuts (4, 6). Therefore, chest nuts are not included in the definition of nuts used in this thesis.

In many Western countries, nuts are consumed as snacks, desserts, are eaten whole, as spreads, oils, or as part of meals or in hidden sources (4, 8). Peanut butter is a particularly popular spread in countries such as the United States and the Netherlands. In Europe, peanut spreads represent about 41% of the total intake of nut spreads, and about 18% of all peanuts are consumed as spread (8). Peanut butter obviously contains many of the nutrients in peanuts, although some additives have been added to enhance its quality, taste, and presentation (6). For example, peanut butter that was sold in the Netherlands in 1986 contained more vitamin B6, sodium, and partially hydrogenated fatty acids, and less niacin than peanuts (6).

Health benefits of nut consumption

Since the establishment of the health claim concerning nut intake and coronary heart disease in 2003, the interest in nuts in the prevention of non-communicable diseases further emerged. Besides the observed inverse associations with cardiovascular diseases, nut intake has also been found to potentially reduce the risk of other age-related chronic disease, like cancer, metabolic syndrome, obesity, type 2 diabetes, hypertension, gallstones, and neurological and psychiatric disorders (4, 7, 9). However, the evidence for these relations

is quite limited and inconsistent. Furthermore, in several meta-analyses of prospective cohort studies, nut intake was found to be associated with reduced total mortality and cause-specific mortality, including deaths due to cancer, cardiovascular disease, respiratory disease, diabetes, and infections (10-12). Nuts might therefore potentially contribute to the prevention of morbidity and mortality related to several chronic diseases, including cancer.

Importance of cancer prevention

Cancer is a huge public health problem worldwide, with approximately 18.1 million incident cancer cases and 9.6 million cancer deaths globally in 2018 (13). Even more worrying, the global burden of cancer is expected to rise to 29.5 million new cancer cases and 16.4 million cancer-related deaths by 2040 due to the growth and ageing of the population (14). Because of the high number of cancer cases and deaths and because of the expected rise in these numbers, we cannot treat our way out of the cancer problem (15). Strategies for the primary prevention of cancer are thus urgently needed, especially for those cancers with a poor prognosis, e.g. cancers of the pancreas, lung and bronchus, liver and intrahepatic bile ducts, esophagus, stomach, brain and other nervous system, and ovaries (16, 17).

Recently, it has been estimated that approximately 42-50% of all cancers can potentially be prevented by avoiding modifiable risk factors, such as cigarette smoking, excess body weight, alcohol intake, unhealthy dietary habits, and physical inactivity (15, 18). Because of the suggested health benefits of nut consumption on chronic diseases, nuts might potentially play a promising role in the chemoprevention of cancer as well.

Nut consumption and cancer: epidemiological evidence

Nut consumption and cancer-related mortality

Several prospective cohort studies investigated the relation between nut consumption and cancer-related mortality. Inverse associations between nut intake and cancer-related mortality were found in a meta-analysis published in 2015, with a pooled hazard ratio (HR) (95% confidence interval (CI)) of 0.86 (0.75-0.98) when comparing the highest to the lowest nut intake category (11). Another meta-analysis published in 2015 included one other prospective cohort study compared to the previous meta-analysis, and also showed that nut intake is inversely associated with cancer-related mortality (HR (95% CI) for highest vs lowest intake category = 0.85 (0.77-0.93)) (12). One year later, a third meta-analysis was published in which the results of nine cohort studies were pooled (10). This resulted in a summary RR (95% CI) per 28 grams increase in nut intake/day of 0.85 (0.76-0.94) (10).

These meta-analyses have consistently observed significant inverse relations between nut intake and cancer-related mortality. However, it is yet unclear whether this inverse relation is also seen for individual cancers as cause of death. Moreover, it is unclear whether it is the result of a positive association between nut intake and cancer prognosis and survival, or of an inverse relation between nut consumption and cancer incidence, i.e. risk.

Nut consumption and cancer risk

A first meta-analysis on the relation between nut consumption and cancer risk was published in 2015 and included 20 case-control studies and 11 cohort studies (19). Overall, nut consumption was found to be significantly associated with a reduced risk of cancer, with a RR for the highest vs the lowest nut intake category (95% CI) of 0.85 (0.76-0.95). Nevertheless, this inverse association was only significant in the case-control studies (RR (95% CI) = 0.82 (0.69-0.98)), not in the cohort studies (HR (95% CI) = 0.91 (0.81-1.02)). This meta-analysis also showed that the association with nut intake differed across cancer types. Significant inverse associations were found between nut consumption and the risk of colorectal, endometrial, and pancreatic cancer. No significant associations with nut consumption were found with the risk of upper-aerodigestive tract cancer, breast cancer, gastric cancer, glioma, hepatocellular carcinoma, leukemia (including acute myeloid leukemia), lymphoma, ovarian cancer, and prostate cancer (19). However, the results for the individual cancer types were based on very few studies; the maximum number of studies per subtype was five for prostate cancer. Moreover, most of the included studies have a case-control design, which is inherently vulnerable to information and selection biases.

This meta-analysis suggested that nuts might provide a promising role in the chemoprevention of certain cancer types, but also demonstrated that studies on nut consumption and cancer risk are very scarce and that the findings are inconclusive. More evidence is needed to be able to more accurately assess the relation between nut intake and individual cancer types, and especially prospective studies to minimize the risk of selection and information biases.

Mechanisms linking nut consumption to cancer risk

Carcinogenesis in a nutshell

Cancer is a disease characterized by autonomous growth of tissues that escaped the mechanisms that normally regulate cell proliferation, cell survival and death, and cell differentiation (20). This disease arises as a result of (somatic) pathological genetic and epigenetic mutations in the genome of the cell (20-22), which may be the result of environmental factors, including biological, chemical and physical carcinogens (20, 22). Mutations accumulate in the cell over time, which makes the cell increasingly genetically unstable, and which may eventually lead to the transformation of a normal cell into a cancer cell (20-22). A cancer cell acquires several abnormal properties: a reduced dependence on signals from other cells for their growth, survival, and division, become less prone to apoptosis, be able to proliferate indefinitely, become genetically unstable with a greatly increased mutation rate, be abnormally invasive, and be able to survive and proliferate in foreign tissues to form secondary tumors (21).

Carcinogenesis, the process during which a normal cell becomes a cancer cell, is a complex process that differs between tumor (sub)types (22). At least four types of genes have been found to play an important role in this process: oncogenes, tumor suppressor genes, DNA repair genes, and telomerase (22). Mutations may transform proto-oncogenes into oncogenes

that are hyperactive, stimulate excessive cell survival and proliferation, or inactivate tumor suppressor genes (21, 22). Tumor suppressor genes normally negatively regulate cell growth, thereby suppressing tumor formation. Mutations or epigenetic changes in these genes may result in a loss of function, which thus may lead to the development of malignant tumors (20-22). Genes involved in DNA damage repair are important to repair errors that occur during DNA replication. Mutations in these genes are not by definition carcinogenic, but cause instability in the genome and result in an increased mutation frequency, which might play an important role in carcinogenesis (22). Lastly, mutations in telomerases, which are involved in the regulation of cell apoptosis, may result in mutated cells escaping apoptosis and further developing into cancerous cells (22).

Potential mechanisms by which nut consumption might reduce cancer risk

As described previously, nuts are very nutrient dense, and the potential mechanisms by which their components might contribute to the prevention of cancer have been investigated. Nevertheless, the working mechanisms have not yet been completely elucidated. Some of the hypothesized mechanisms are described below.

One of the most frequently mentioned mechanisms relates to the antioxidant activity of nut components. Antioxidants in nuts prevent oxidative stress and DNA damage by reactive oxygen species, which develop by internal or external factors (4, 5, 9, 23-39). Components with antioxidant capacity include vitamins A, B (folic acid), and E, selenium, magnesium, zinc, melatonin, omega-3 polyunsaturated fatty acids, phytosterols, and several polyphenols (4, 5, 9, 23-39). Secondly, vitamins B and E, magnesium, selenium, L-arginine, fiber, polyunsaturated fatty acids, phytosterols, and polyphenols (especially quercetin and resveratrol) may regulate the inflammatory response and immunological activity by influencing the formation and metabolism of prostaglandins and pro-inflammatory cytokines, which are often involved in carcinogenesis (4, 5, 9, 23, 25-33, 35-42). Some nut components might influence the regulation of cell transformation, differentiation, and proliferation, such as vitamins B and E, magnesium, zinc, omega-3 and omega-6 polyunsaturated fatty acids and several polyphenols (5, 9, 23-30, 33, 35, 36, 38, 40, 42, 43). Moreover, mono- and polyunsaturated fatty acids and polyphenols in nuts may also reduce tumor initiation and promotion (5, 23, 26, 27, 30, 32, 33, 35, 38-41, 44). Vitamin E, inositol polyphosphates, mono- and polyunsaturated fatty acids, and several polyphenols have also been found to modulate angiogenesis, which is essential for the blood supply of growing solid tumors (5, 24, 29, 31, 33, 38, 40-42). In addition, the polyphenols quercetin and resveratrol inhibit chemically-induced carcinogenesis (9). Omega-3 polyunsaturated fatty acids and several polyphenols induce detoxifying metabolic enzymes, thereby preventing cell damage (9, 23, 27, 31-33, 35-37, 44). Vitamin B (folic acid) and magnesium may reduce DNA damage or induce DNA damage repair, which is important in the maintenance of DNA synthesis and integrity. Folic acid is a coenzyme that is involved in the synthesis of nucleic acids and the metabolism of amino acids (4, 9, 23, 28, 35, 43). Furthermore, apoptosis is regulated or induced by vitamin E, magnesium, zinc, inositol polyphosphates, phytosterols, omega-3 and omega-6 polyunsaturated fatty acids, and polyphenols, thereby potentially limiting the survival of cancer cells (5, 25, 26, 28-30, 33, 35, 36, 38, 40, 42, 44). Vitamin B (B6, folic acid), copper,

magnesium, phytosterols, fiber, mono- and poly-unsaturated fatty acids, and polyphenols alter lipid profiles and cell metabolism (5, 9, 23, 26, 27, 32, 39). Because reprogramming of energy metabolism is a key feature of cancer cells, this might contribute to the prevention of cancer (5). In addition, especially two groups of phytoestrogens (isoflavonoids and lignans), but also other polyphenols, phytosterols, and some fatty acids modify hormonal mechanisms or conduct antiestrogenic activity, which may contribute to the prevention of hormone-dependent cancers (5, 9, 23-26, 30, 33, 35, 36, 40). Lastly, fiber in nuts may not only have metabolic effects, but also increase fecal volume and anaerobic fermentation. Consequently, carcinogens are diluted in the gut and the intestinal transit time is reduced. This may especially be important in the prevention of colorectal cancer (9, 23, 43).

Rationale and aim of this thesis

Because of the high and increasing number of cancer diagnoses each year and the poor prognosis of some cancer types, it is important to focus on preventive strategies. Despite the increasing interest in nuts because of the new promising insights in their potential cancer preventive properties, the evidence for an association between nut intake and cancer risk is limited and requires confirmation in prospective studies. Therefore, the primary aim of this thesis was to investigate the associations between nut and peanut butter intake and the risk of cancer in men and women in the prospective Netherlands Cohort Study on diet and cancer (NLCS) (45). More specifically, we studied the associations of total nut, tree nut, peanut, and peanut butter intake with the risk of esophageal, gastric, colorectal, pancreatic, lung, breast, endometrial, ovarian, prostate, and total cancer in men and women. Moreover, we investigated whether these associations are nonlinear, whether they differ across subtypes of the above-mentioned cancers, and whether they are modified by sex, smoking, alcohol intake, BMI, physical activity, and educational level.

As secondary aim, we investigated the associations between nut and peanut butter intake and the risk of colorectal tumors harboring *APC*, *KRAS*, or *BRAF* mutations, p53 overexpression, or microsatellite instability, to account for molecular heterogeneity and to provide new insights in the possible involvement of these genes in the associations between nut intake and colorectal cancer development.

Study design and population

All studies included in this thesis were performed within the prospective NLCS (45). In the NLCS, 120,852 men and women aged 55-69 years at baseline were included in September 1986. At baseline, all participants filled in a self-administered 11-page baseline questionnaire on dietary habits, lifestyle, and other potential cancer risk factors. This baseline questionnaire included a validated 150-item food frequency questionnaire (FFQ) that focused on habitual food consumption during the year preceding baseline (46). The NLCS was approved by the institutional review boards of the Maastricht University and the Netherlands Organization for Applied Scientific Research TNO.

To improve the efficiency of the data collection and processing, a case-cohort approach was applied. In this design, cancer cases were derived from the entire cohort, whereas the person-years at risk for the total cohort were estimated using a subcohort. This subcohort consisted of 5000 participants who were randomly sampled from the entire cohort at baseline, and was followed-up biennially for information on vital status. Cancer cases in the total cohort were identified through annual record linkage with the Netherlands Cancer Registry and the Netherlands Pathology Registry (PALGA) (47).

In this thesis, we used data from the follow-up period September 1986-December 2006, the maximum follow-up period that was available at the start of this project. The follow-up period September 1986-December 1993 (and excluding the first 2.3 years) was used for the analysis of the molecular subtypes of colorectal cancer, because tumor material has been collected for colorectal tumors that occurred during this period.

Outline of the thesis

The chapters in this thesis are ordered based on the topographic sites of the cancers according to ICD-O-3 codes, starting with gastrointestinal cancers, followed by other cancers and total cancer. This thesis starts with the study on the associations of nut and peanut butter consumption with the risk of esophageal and gastric cancer in Chapter 2 and with the risk of colorectal cancer and its anatomical and molecular subtypes in Chapter 3. In Chapter 4, the associations between nut and peanut butter intake and pancreatic cancer risk were investigated. Chapter 5 describes the associations of nut and peanut butter intake with the risk of lung cancer and its four major histologic subtypes. The associations of nut and peanut butter intake and the risk of postmenopausal breast cancer and its estrogen/progesterone receptor subtypes are described in Chapter 6, and the associations with endometrial and ovarian cancer risk in Chapter 7. Chapter 8 describes the relations between nut and peanut butter intake and risk of total, advanced, and non-advanced prostate cancer. Furthermore, Chapter 9 presents the study on the associations between nut consumption and the risk of total cancer and of smoking- and alcohol-related cancer subgroups. Finally, Chapter 10 provides a general discussion of the main findings described in this thesis.

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Chapter 2

Tree nut, peanut, and peanut butter consumption and the risk of gastric and esophageal cancer subtypes: The Netherlands Cohort Study

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Gastric Cancer. 2018; 21: 900-912

Abstract

Background: Nut consumption has been associated with reduced cancer-related mortality. However, it is unclear whether nut consumption also reduces the risk of esophageal and gastric cancer subtypes. We prospectively investigated the relationship of tree nut, peanut, and peanut butter intake with risk of esophageal squamous cell carcinoma (ESCC), esophageal adenocarcinoma (EAC), gastric cardia adenocarcinoma (GCA), and gastric non-cardia adenocarcinoma (GNCA) in the Netherlands Cohort Study.

Methods: In 1986, 120,852 males and females, aged 55-69 years, completed a baseline questionnaire on diet and cancer risk factors. After 20.3 years of follow-up, 133 ESCC, 200 EAC, 191 GCA, and 586 GNCA cases, and 3,720 subcohort members were available for multivariable Cox regression analyses, using a case-cohort approach.

Results: Increased total nut consumption was significantly associated with a decreased risk of ESCC and GNCA (HRs (95% CIs) for 10+ g/day vs. nonconsumers = 0.54 (0.30-0.96) and 0.73 (0.55-0.97), respectively), but not with EAC and GCA risk. Similar trends were observed for tree nut and peanut intake, which were mostly nonsignificant. For peanut butter intake, no significant associations were found. When excluding the first four years of follow-up to reduce the possible influence of reversed causation, the relation between nut consumption and ESCC risk attenuated, but remained inverse.

Conclusions: Our findings suggest that increased tree nut and peanut consumption is inversely associated with GNCA risk and possibly with ESCC risk, but not with the risk of the other esophageal and gastric cancer subtypes.

Introduction

In the past few years, the interest in nuts has been increasing because of their perceived health benefits. Besides other health advantages, recent meta-analyses have demonstrated that increased nut consumption may lower the risk of cancer and cancer-related mortality [1-4]. Nuts are nutrient dense foods and contain vitamins, minerals, mono- and polyunsaturated fatty acids, polyphenols, and several other compounds that might act as cancer-chemopreventive agents [5, 6].

Currently, little evidence is available on the relation between nut consumption and the risk of esophageal and gastric cancer, while these two cancers were the sixth (esophageal) and third (gastric) most common causes of death from cancer globally in 2012 [7]. Based on histologic and topographic subtyping, esophageal and gastric cancer can be subdivided into esophageal squamous cell carcinoma (ESCC), esophageal adenocarcinoma (EAC), gastric cardia adenocarcinoma (GCA), and gastric non-cardia adenocarcinoma (GNCA). A growing number of studies indicates that these subtypes differ regarding their etiologies and risk factors [8, 9]. Special interest is in EAC and GCA, because the incidence rates of these subtypes have increased considerably in the US and in many European countries in the past decades [10, 11].

In a recently published prospective cohort study, nut and peanut butter consumption was significantly inversely associated with GNCA risk, but not with risk of the other esophageal and gastric cancer subtypes [12]. Unfortunately, the authors could not analyze types of nuts separately. Moreover, results from four case-control studies investigating the association between nut consumption and gastric cancer are inconclusive [13-16], and one case-control study found an inverse association between peanut consumption and ESCC risk [17]. Another case-control study observed no association between fruit and nut consumption combined and upper aerodigestive tract cancer risk [18].

In this study, we prospectively investigated the associations of tree nut, peanut, and peanut butter consumption with the risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study (NLCS). Moreover, we investigated the exposure-response curves and whether the associations were modified by sex, alcohol consumption, cigarette smoking, educational level, and body mass index (BMI).

Methods

Study design and cancer follow-up

In this analysis, data from the NLCS were used. In September 1986, 62,573 females and 58,279 males, aged 55-69 years, completed a mailed, self-administered baseline questionnaire on cancer risk factors [19]. The institutional review boards from the Maastricht University and the Netherlands Organization for Applied Scientific Research approved the NLCS. By filling in and returning the baseline questionnaire, participants agreed to participate in the study.

For data processing and analysis, a case-cohort approach was used for efficiency reasons. A subcohort (n = 5,000) was randomly sampled from the total cohort immediately after baseline, and accumulated person-years were estimated from this subcohort. Vital status information of subcohort members was obtained biennially from 17 September 1986 until 1 January 2007, and was 100% complete after 20.3 years. Incident cancer cases in the total cohort were detected through annual record linkage with the Netherlands Cancer Registry and the nationwide Dutch Pathology Registry (PALGA) [20]. The completeness of cancer incidence follow-up is estimated to be more than 95% [21].

During 20.3 years of follow-up, 164 ESCC, 259 EAC, 254 GCA, and 741 GNCA cases without prevalent cancer (except skin cancer) at baseline were detected. Histology codes for esophageal cancer (ICD-O-3 code C15) included 8050-8076 for ESCC, and 8140-8141, 8190-8231, 8260-8263, 8310, 8430, 8480-8481, 8490, 8560, and 8570-8572 for EAC. For gastric cancer (ICD-O-3 code C16), histology codes were C16.0 for GCA and C16.1-16.9 for GNCA. Participants with incomplete or inconsistent dietary data were excluded from the analysis, as were participants with missing values on predefined confounders. In the current analysis, 133 ESCC, 200 EAC, 191 GCA, and 586 GNCA cases, and 3,720 subcohort members were included (Fig. 1).

Exposure assessment

The 11-page mailed, self-administered baseline questionnaire consisted of questions regarding diet, smoking habits, anthropometry, disease history, physical activity, and other cancer risk factors [19]. Information on diet was obtained from a validated 150-item semi-quantitative food-frequency questionnaire (FFQ) that asked about habitual diet in the year before baseline [22]. Intake of tree nuts, peanuts, and peanut butter was assessed by measuring the number of standard portion sizes consumed per intake and the intake frequencies of 'peanuts', 'other, mixed nuts' (tree nuts), and 'peanut butter'. Frequency categories could range from 'never or less than 1x/month' to '6-7x/week'. A standard portion size of peanuts or tree nuts was assumed to be 28 grams, and one portion of peanut butter 15 grams per slice of bread. Mean daily intake was calculated in grams, by multiplying intake frequencies and portion sizes. Total nut consumption was calculated as the sum of tree nut and peanut intake.

Statistical analysis

The associations between nut intake and risk of esophageal and gastric cancer subtypes were analyzed with age- and sex-adjusted and multivariable-adjusted Cox proportional hazards models. The robust Huber-White sandwich estimator was used to estimate standard errors that take into account the additional variance introduced by sampling from the total cohort [23]. The proportional hazards assumption was checked with Schoenfeld residuals and –ln(-ln) survival plots [24]. If the assumption was violated for a variable, the interaction between that variable and time was tested by including a time-varying covariate in the model.

The associations between nut and peanut butter intake and risk of esophageal and gastric

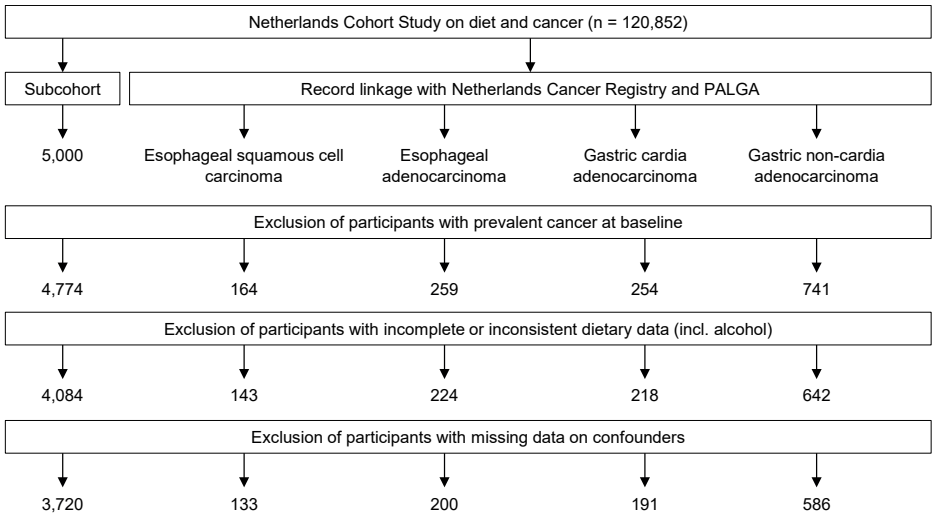

Fig. 1 Flow diagram of the number of subcohort members and gastric and esophageal cancer cases on whom the analyses were based

cancer subtypes were investigated on a categorical and continuous scale in survival analyses. We combined both sexes in the categorical analyses, because of the limited number of cases and because no statistically significant interaction by sex was found (Supplementary Table 2). In the continuous analyses, we additionally estimated hazard ratios (HR) for males and females separately. For the categorical analyses, total nut and peanut consumption were divided into four categories of 0, 0.1-<5, 5-<10, and 10+ g/day. Due to the smaller number of cases in the higher intake categories, tree nut consumption was categorized into 0 and 0.1+ g/day, and peanut butter into 0, 0.1-<5, and 5+ g/day. The category of nonconsumers formed the reference group. Tests for trends were performed by assigning sex-specific median values of nut intake in the subcohort to the intake categories and fitting these as continuous terms in the regression models. In the continuous analyses, HRs were estimated per increment of 5 g/day.

In multivariable-adjusted survival analyses, the associations were corrected for the following predefined confounders, which were included in the final multivariable-adjusted model independent of their effect on the estimated HRs: age at baseline (years; continuous), sex (male, female), cigarette smoking (status (never, former, current), frequency (number of cigarettes per day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5, 18.5-<25, 25-<30, and 30+ kg/m^2), nonoccupational physical activity (≤30, >30-≤60, >60-≤90, >90 min/day), highest level of education (primary school or lower vocational (low), secondary school or medium vocational (medium), and higher vocational or university (high)), total energy intake (kcal/day; continuous), alcohol consumption (0, 0.1-<5, 5-<15, 15-<30, and 30+ g/day), and family history of esophageal (for esophageal cancer subtypes) or gastric cancer (for gastric cancer subtypes). Other potential confounders considered were: intake of fruits, vegetables, tea, coffee, red meat, processed meat, fish, and total salt,

history of gastric ulcers, and long term use (>6 months) of nonsteroidal anti-inflammatory drugs (including aspirin) and lower esophageal sphincter-relaxing medications. However, because these potential confounders did not change the HRs with 10% when using a backward stepwise selection procedure, only the predefined confounders were included in the final multivariable-adjusted model.

Tests for heterogeneity were performed to investigate etiologic differences between the four major subtypes of esophageal and gastric cancer using a competing risk procedure. In this analysis, a bootstrapping method developed for the case-cohort approach was used to estimate the standard errors for the observed differences in associations [25, 26].

The linearity of the exposure-response relation between nut intake and risk of esophageal and gastric cancer subtypes was assessed in restricted cubic spline analyses, in models with three fixed knots at 0, 5, and 10 g nut intake/day. Because no assumptions are made about the shape of the relation between the exposure and outcome variables in restricted cubic spline analysis, it is a useful method to test for nonlinearity and to present nonlinear relationships. Detailed sensitivity analyses were performed to investigate whether choosing additional knots or other knot positions would improve the model fit, as measured with the Akaike Information Criterion (AIC) score [27].

To investigate possible interactions by esophageal and gastric cancer risk factors, categorical analyses of nut consumption were performed stratified by sex, baseline BMI, smoking status, alcohol consumption, and educational level. To increase statistical power, the two highest nut intake categories were merged. Interactions were tested by including cross-product terms in the models and performing Wald tests.

To check for potential reversed causation due to preclinical cancer at baseline, we divided the total follow-up time in 4-year periods and compared the median nut intake at baseline of cases diagnosed during these periods. A Kruskal-Wallis test was performed to test the statistical significance of a possible difference in median nut intake. Moreover, we repeated the Cox regression analyses after excluding the first four years of follow-up. Additionally, we restricted the analyses to cases who had stated having had a constant peanut butter intake during the five years before baseline. We do not have these data for tree nut or peanut consumption.

In sensitivity analyses, we additionally adjusted for adherence to the Mediterranean diet, by including the alternate Mediterranean diet score (aMed) into the regression models [28]. Since nuts comprise one of the components of the aMed score, and because alcohol consumption is positively associated with esophageal and gastric cancer, an adapted version was used (excluding nuts and alcohol), which ranged from 0 (no adherence) to 7 (maximal adherence).

All analyses were performed in Stata 14 software (StataCorp. 2015. College Station, TX). *P* values were tested two-sided, and were considered statistically significant if $p<0.05$.

Table 1 Baseline characteristics (mean (SD) or percent) of cases and subcohort members in the Netherlands Cohort Study on diet and cancer, 1986-2006

	Men					Women				
	Subcohort[a]	Cases[a]				Subcohort[a]	Cases[a]			
	(N=1,834)	ESCC (N=76)	EAC (N=157)	GCA (N=158)	GNCA (N=390)	(N=1,886)	ESCC (N=57)	EAC (N=43)	GCA (N=33)	GNCA (N=196)
Age (years)	61.2 (4.2)	61.8 (4.1)	61.2 (4.0)	61.2 (4.2)	62.1 (4.1)	61.4 (4.2)	62.5 (4.2)	62.1 (4.4)	61.8 (4.0)	62.4 (4.2)
BMI (kg/m^2)	24.9 (2.6)	24.4 (3.1)	25.6 (2.5)	25.5 (2.6)	24.8 (2.8)	25.0 (3.5)	24.1 (3.6)	26.9 (4.3)	26.5 (4.3)	25.3 (3.9)
Never cigarette smokers (%)	13.6	9.2	8.3	10.1	9.5	58.9	35.1	62.8	45.5	52.0
University or higher vocational education (%)	20.3	19.7	19.8	21.5	12.3	9.5	5.3	7.0	15.2	6.6
Nonoccupational physical activity (min/d)	81.0 (67.4)	72.9 (66.7)	80.1 (62.2)	86.8 (78.3)	86.1 (75.1)	65.5 (50.6)	61.9 (60.6)	66.8 (58.7)	70.6 (66.6)	65.2 (54.6)
Family history of esophageal cancer (%)	0.8	0.0	1.3	1.3	1.3	0.9	3.5	7.0	0.0	1.0
Family history of gastric cancer (%)	6.9	9.2	7.6	8.9	11.5	6.4	5.3	4.7	6.1	11.7
History of gastric ulcers (%)	12.1	21.1	12.1	10.8	25.6	4.5	7.0	7.0	6.1	8.7
Long term use (>0.5 year) of nonsteroidal anti-inflammatory drugs (including aspirin) (%)	5.3	4.0	7.0	4.4	3.6	7.6	8.8	7.0	12.1	7.1
Long-term use (>0.5 year) of lower esophageal sphincter-relaxing medications (%)	15.4	14.5	21.0	17.1	17.4	14.0	12.3	9.3	15.2	13.8
Food intake										
Total nuts (g/day)	7.9 (13.7)	7.8 (15.9)	9.6 (17.3)	7.6 (12.8)	6.7 (11.6)	4.4 (8.5)	3.1 (5.3)	7.3 (13.1)	3.3 (4.6)	3.1 (6.7)
Tree nuts (g/day)	1.0 (3.4)	0.4 (1.0)	1.2 (3.6)	0.7 (2.3)	0.8 (2.5)	1.1 (4.0)	0.7 (2.0)	1.2 (5.5)	0.4 (0.7)	0.5 (1.1)
Peanuts (g/day)	6.9 (13.0)	7.4 (15.8)	8.3 (16.2)	6.9 (12.2)	5.9 (10.6)	3.3 (7.0)	2.4 (4.1)	6.1 (12.3)	2.7 (4.2)	2.6 (6.4)
Peanut butter (g/day)	1.4 (4.2)	2.0 (6.0)	2.2 (5.9)	1.2 (3.5)	1.2 (3.0)	1.2 (3.6)	1.1 (3.1)	0.5 (1.6)	0.2 (0.5)	1.0 (3.5)
Energy (kcal/day)	2167 (499)	2045 (463)	2181 (473)	2130 (502)	2193 (513)	1684 (389)	1749 (389)	1639 (417)	1807 (341)	1675 (378)
Alcohol (g/day)	15.1 (17.1)	29.0 (28.7)	16.5 (17.3)	16.2 (15.8)	15.6 (17.6)	6.0 (9.5)	12.8 (17.5)	6.1 (8.4)	4.9 (9.2)	5.8 (9.5)
Fruit (g/day)	156.4 (115.3)	102.7 (83.2)	167.7 (131.4)	145.0 (111.6)	146.9 (115.3)	195.3 (118.3)	177.8 (120.6)	218.5 (105.8)	199.1 (126.8)	195.6 (133.6)
Vegetables (g/day)	187.4 (75.5)	165.3 (67.3)	188.4 (79.2)	186.0 (80.7)	181.9 (76.2)	192.1 (75.1)	197.4 (60.5)	167.7 (60.6)	205.7 (80.4)	190.7 (80.0)
Red meat (g/day)	93.5 (41.3)	98.8 (37.5)	94.1 (38.9)	94.4 (42.6)	95.8 (38.4)	80.8 (37.8)	83.9 (39.9)	84.1 (36.2)	88.5 (46.2)	82.5 (42.6)
Processed meat (g/day)	15.8 (16.8)	17.1 (16.3)	16.5 (16.7)	16.0 (14.7)	17.1 (16.9)	10.3 (11.5)	9.2 (10.2)	7.7 (10.2)	10.6 (12.6)	10.1 (11.5)
Fish (g/day)	13.8 (16.1)	12.9 (14.0)	14.1 (17.4)	15.8 (20.0)	13.7 (17.6)	11.3 (13.2)	14.0 (13.9)	12.0 (16.0)	8.6 (11.1)	11.5 (16.3)
Coffee (cups/day)	4.5 (2.3)	4.7 (2.1)	5.0 (2.5)	5.0 (2.1)	5.0 (2.3)	4.0 (2.0)	4.3 (2.3)	4.2 (1.7)	4.3 (2.2)	4.0 (1.9)
Tea (cups/day)	2.5 (2.0)	2.1 (1.8)	2.6 (2.2)	2.2 (1.9)	2.6 (1.9)	3.0 (2.1)	3.3 (2.7)	2.8 (2.5)	2.9 (2.3)	3.0 (2.1)
Total salt (g/day)	8.8 (3.1)	9.4 (3.7)	8.6 (3.2)	8.7 (3.4)	9.0 (3.6)	8.6 (2.9)	8.9 (3.0)	7.8 (2.1)	8.4 (3.2)	8.5 (2.5)

ESCC, esophageal squamous cell carcinoma; EAC, esophageal adenocarcinoma; GCA, gastric cardia adenocarcinoma; GNCA, gastric non-cardia adenocarcinoma

[a] Number of cases and subcohort members excluding participants with incomplete or inconsistent dietary data (including alcohol consumption) or missing values on predefined confounder variables.

Results

Table 1 shows the baseline characteristics of subcohort members and cases for males and females separately. All cases, except EAC cases, consumed on average less total nuts and tree nuts than subcohort members in both sexes. Male subcohort members consumed less peanuts than esophageal cancer cases, but more than GNCA cases. Female subcohort members consumed more peanuts than all cases, except EAC cases. Moreover, in males, subcohort members consumed more peanut butter than gastric cancer cases, but less than esophageal cancer cases. In females, subcohort members consumed more peanut butter than cases.

Cases and subcohort members also differed with respect to other, potentially confounding factors: compared to subcohort members, cases of both sexes were older (except for male EAC and GCA cases), more often ever smokers (except for female EAC cases), and lower educated (except for GCA cases). EAC and GCA cases of both sexes and female GNCA cases were heavier than subcohort members. Moreover, male cases more often reported a positive family history of gastric cancer than subcohort members, and female cases more often reported gastric ulcers. Furthermore, male cases consumed more alcohol, red and processed meat, and coffee than subcohort members. Female cases also consumed more red meat and coffee than subcohort members.

In Table 2, multivariable-adjusted associations between nut and peanut butter consumption and risk of esophageal and gastric cancer subtypes are presented. The proportional hazards assumption was potentially violated for some categories of total nut and peanut consumption in the analyses of ESCC and GNCA risk, and for peanut butter consumption in the analyses of ESCC and EAC risk. Age- and sex-adjusted results are presented in Supplementary Table 1, which were not importantly different from the multivariable-adjusted results: positive associations became slightly stronger, whereas inverse associations became somewhat weaker after multivariable-adjustment.

For total nut consumption, a statistically significant inverse relation was found with ESCC risk: the HR (95% CI) for those consuming 10+ g/day versus nonconsumers was 0.54 (0.30-0.96) (P_{trend} = 0.050). Increased total nut intake was non-significantly positively related to EAC risk (P_{trend} = 0.578), and, in continuous analyses, a significant positive association with EAC risk was found in women only (HR (95% CI) per 5 g/day increment = 1.19 (1.07-1.32)). No clear relation with total nut intake was seen for GCA risk. For GNCA risk, a nonsignificant inverse trend was found (P_{trend} = 0.088), with significant inverse associations in all total nut consumption categories: the HRs (95% CI) for those consuming 0.1-<5, 5-<10, and 10+ g total nuts/day vs. nonconsumers were 0.79 (0.63-0.98), 0.62 (0.44-0.87), and 0.73 (0.55-0.97), respectively. No significant interaction between total nut consumption categories and sex was observed for the esophageal and gastric cancer subtypes ($P_{interaction}$ ≥0.206) (Supplementary Table 2). In sensitivity analyses, additional adjustment for the aMed score did not importantly alter the results (data not shown).

Table 2 Multivariable-adjusted HRs (95% CIs) for risk of esophageal and gastric cancer subtypes according to nut intake; Netherlands Cohort Study on diet and cancer, 1986-2006

		Subcohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric non-cardia adenocarcinoma	
	Median[a]	Person time at risk (years)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)
Total nut consumption (g/day)										
0.0	0.0	22,062	59	1 (ref)	60	1 (ref)	61	1 (ref)	252	1 (ref)
0.1-<5	2.5	21,821	42	0.81 (0.52-1.24)[c]	68	1.23 (0.85-1.78)	72	1.18 (0.81-1.71)	184	0.79 (0.63-0.98)[d]
5-<10	8.2	7,753	15	0.78 (0.42-1.46)[c]	29	1.39 (0.85-2.26)	22	0.91 (0.53-1.57)	51	0.62 (0.44-0.87)
10+	19.9	11,269	17	0.54 (0.30-0.96)	43	1.23 (0.78 -1.93)	36	0.89 (0.56-1.42)	99	0.73 (0.55-0.97)[c]
P_{trend}				0.050		0.578		0.369		0.088
Continuous, per 5 g/day increment										
Overall		62,905	133	0.97 (0.88-1.07)	200	1.05 (1.00-1.10)	191	0.98 (0.92-1.05)	586	0.96 (0.91-1.00)[c]
Men		29,250	76	1.01 (0.92-1.11)	157	1.03 (0.98-1.09)	158	0.99 (0.93-1.05)	390	0.97 (0.92-1.02)
Women		33,655	57	0.82 (0.66-1.03)	43	1.19 (1.07-1.32)	33	0.84 (0.63-1.12)	196	0.88 (0.76-1.02)
Tree nut consumption (g/day)										
0.0	0.0	44,547	110	1 (ref)	145	1 (ref)	142	1 (ref)	455	1 (ref)
0.1+	2.1	18,358	23	0.56 (0.35-0.90)	55	1.07 (0.77-1.49)	49	0.94 (0.66-1.35)	131	0.82 (0.66-1.02)
Continuous, per 5 g/day increment										
Overall		62,905	133	0.62 (0.37-1.04)	200	1.09 (0.95-1.24)	191	0.72 (0.46-1.14)	586	0.81 (0.66-0.99)
Men		29,250	76	0.46 (0.20-1.07)	157	1.08 (0.93-1.25)	158	0.79 (0.50-1.23)	390	0.89 (0.73-1.09)[d]
Women		33,655	57	0.71 (0.38-1.33)[d]	43	1.15 (0.87-1.52)[c]	33	0.23 (0.06-0.90)	196	0.52 (0.33-0.81)
Peanut consumption (g/day)										
0.0	0.0	25,552	63	1 (ref)	71	1 (ref)	70	1 (ref)	269	1 (ref)
0.1-<5	2.5	22,960	43	0.83 (0.54-1.26)[c]	70	1.11 (0.77-1.60)	71	1.05 (0.74-1.51)	192	0.84 (0.68-1.05)[d]
5-<10	8.5	5,762	13	0.90 (0.47-1.75)[d]	23	1.30 (0.76-2.22)	17	0.85 (0.47-1.52)	43	0.69 (0.48-0.99)
10+	21.4	8,630	14	0.62 (0.33-1.15)	36	1.21 (0.75-1.94)	33	1.00 (0.62-1.61)	82	0.84 (0.63-1.13)[c]
P_{trend}				0.185		0.508		0.869		0.351

(continued)		Subcohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric non-cardia adenocarcinoma	
	Median[a]	Person time at risk (years)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)
Continuous, per 5 g/day increment										
Overall		62,905	133	0.99 (0.90-1.08)	200	1.04 (0.99-1.10)	191	0.99 (0.94-1.06)	586	0.97 (0.92-1.02)[c]
Men		29,250	76	1.03 (0.94-1.12)	157	1.03 (0.97-1.09)	158	1.00 (0.94-1.06)	390	0.97 (0.92-1.02)[c]
Women		33,655	57	0.83 (0.66-1.05)	43	1.20 (1.07-1.35)	33	0.88 (0.64-1.21)	196	0.93 (0.79-1.08)
Peanut butter consumption (g/day)										
0.0	0.0	45,489	98	1 (ref)	140	1 (ref)	139	1 (ref)	433	1 (ref)
0.1-<5	1.2	10,970	20	1.06 (0.64-1.75)[d]	37	1.21 (0.82-1.78)[c]	37	1.17 (0.79-1.72)	101	1.09 (0.85-1.39)
5+	9.6	6,446	15	1.47 (0.81-2.67)	23	1.26 (0.78-2.04)[d]	15	0.78 (0.44-1.37)	52	0.88 (0.63-1.21)
P_{trend}				0.190		0.248		0.491		0.474
Continuous, per 5 g/day increment										
Overall		62,905	133	1.25 (1.03-1.52)	200	1.15 (1.00-1.33)	191	0.90 (0.70-1.15)	586	0.93 (0.81-1.06)
Men		29,250	76	1.33 (1.06-1.67)[d]	157	1.19 (1.03-1.37)	158	0.98 (0.79-1.22)	390	0.92 (0.79-1.07)
Women		33,655	57	1.09 (0.75-1.58)	43	0.68 (0.33-1.39)	33	0.08 (0.01-0.77)	196	0.94 (0.71-1.24)

[a] Median in the subcohort

[b] Adjusted for age (years; continuous), sex, cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5/18.5-<25/25-<30/30+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of esophageal cancer (for esophageal cancer subtypes; no/yes), family history of gastric cancer (for gastric cancer subtypes; no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

[c] The proportional hazards assumption was violated for the exposure variable, and there was a statistically significant interaction with time

[d] The proportional hazards assumption was violated for the exposure variable, but there was no statistically significant interaction with time

The heterogeneity test for the relation of categorical total nut intake with risk of esophageal and gastric cancer subtypes was significant ($P_{heterogeneity}$ = 0.008). To further investigate this finding, we compared the subtypes pairwise. Significant differences in associations were only found between EAC and GNCA ($P_{heterogeneity}$ = 0.004) and between GCA and GNCA ($P_{heterogeneity}$ = 0.049). A borderline significant difference was observed between ESCC and EAC ($P_{heterogeneity}$ = 0.050).

Tree nut intake was significantly associated with a lower risk of ESCC (HR (95% CI) for consumers vs. nonconsumers = 0.56 (0.35-0.90)). For EAC risk, a nonsignificant positive association was found. Tree nut consumption was non-significantly inversely related to GCA risk, and the HR (95% CI) per 5 g/day increment was 0.23 (0.06-0.90) in women in continuous analyses. For GNCA risk, also a nonsignificant inverse association was observed, and, in continuous analyses, significant inverse associations in the overall population and in women: the HRs (95% CI) per 5 g/day increment were 0.81 (0.66-0.99) and 0.52 (0.33-0.81), respectively.

For peanut intake, nonsignificant inverse associations with risk of ESCC and GNCA were found, and a HR (95% CI) for GNCA for those consuming 5-<10 g peanuts/day compared to nonconsumers of 0.69 (0.48-0.99). The risk of EAC was non-significantly increased in participants with a higher peanut intake, and this association was significant in women in continuous analyses (HR (95% CI) per 5 g/day increment = 1.20 (1.07-1.35)). For GCA risk, no association with peanut intake was found.

Increasing peanut butter intake was associated with a non-significantly increased risk of ESCC and EAC. In continuous analyses, the association with ESCC risk was significant in the overall population and in men (HR (95% CI) = 1.25 (1.03-1.25) and 1.33 (1.06-1.67), respectively), as was the association with EAC risk in men (HR (95% CI) per 5 g/day increment = 1.19 (1.03-1.37)). Unclear associations were found between peanut butter consumption and GCA and GNCA risk, although a significant inverse association with GCA risk was observed in women in continuous analyses (HR (95% CI) per 5 g/day increment = 0.08 (0.01-0.77)).

Fig. 2 presents the restricted cubic spline curves with three fixed knots at 0, 5, and 10 g nut intake/day for the risk of (a) ESCC, (b) EAC, (c) GCA, and (d) GNCA according to total nut consumption. A clear leveling-off of ESCC, GCA, and GNCA risk can be seen for total nut intake of more than 5 g/day. Statistical evidence for nonlinearity was only found for GNCA ($P_{nonlinearity}$ = 0.001). For tree nut, peanut, and peanut butter intake, the test for nonlinearity was solely significant for the relation between peanut consumption and GNCA risk ($P_{nonlinearity}$ = 0.013). Choosing additional knots or other knot positions did not improve the fit of the model, as measured with the AIC score (data not shown).

In the stratified analyses, we observed no significant interactions between total nut consumption categories and potential risk factors for ESCC, GCA, and GNCA (Supplementary Table 2). For EAC, we found a significant interaction between total nut intake and educational level ($P_{interaction}$ = 0.043), with positive associations in the subgroups with a low or medium

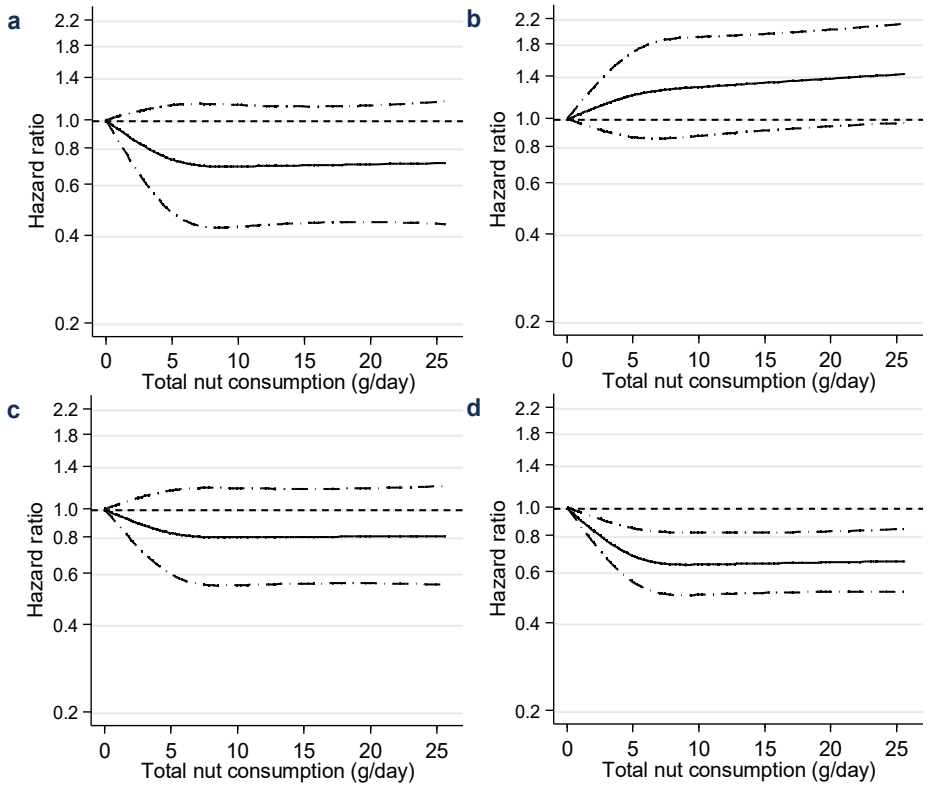

Fig. 2 Multivariable-adjusted restricted cubic spline curves for the association between total nut consumption (g/day) and risk of **(a)** esophageal squamous cell carcinoma (ESCC), **(b)** esophageal adenocarcinoma (EAC), **(c)** gastric cardia adenocarcinoma (GCA), and **(d)** gastric non-cardia adenocarcinoma (GNCA). Solid lines represent point estimates; dashed lines represent 95% CIs. *P* values for nonlinearity were 0.193 for ESCC, 0.394 for EAC, 0.299 for GCA, and 0.001 for GNCA. HRs were adjusted for age (years; continuous), sex, cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5/18.5-<25/25-<30/30+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of esophageal cancer (for esophageal cancer subtypes; no/yes), family history of gastric cancer (for gastric cancer subtypes; no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

educational level, and an unclear trend in the subgroup with a high educational level (Supplementary Table 3). However, because of the low case numbers and the large number of Wald tests performed, this finding may be due to chance.

The median total nut intake at baseline of ESCC cases diagnosed earlier in the follow-up was lower than that of cases diagnosed later in time (Supplementary Table 4), and the Kruskal-Wallis test showed that there was a significant difference between the 4-year periods (*P* = 0.019). For GNCA, the median intake was higher in earlier versus later cases, although not significantly (*P* = 0.077). For EAC and GCA cases, no significant differences were observed.

Table 3 presents the multivariable-adjusted HRs for risk of esophageal or gastric cancer subtypes according to nut intake after excluding the first four years of follow-up. When excluding the first four years of follow-up, most inverse associations of categorical nut and peanut butter intake with ESCC risk attenuated: e.g. the HR (95% CI) for ESCC for 0.1-<5 g total nut intake/day vs. nonconsumers changed from 0.81 (0.52-1.24) to 1.05 (0.66-1.66), and that for 5-<10 g total nut consumption/day vs. nonconsumers from 0.78 (0.42-1.46) to 0.95 (0.49-1.84). For EAC, GCA, and GNCA risk, the associations were essentially the same as when the total follow-up period was included. Restricting the analysis of peanut butter to only those participants with a constant peanut butter intake during the five years before baseline did not importantly change the results (data not shown).

Discussion

In this prospective cohort study, increased total nut consumption was significantly associated with a decreased risk of ESCC and GNCA. A nonsignificant positive association was observed with EAC risk and no clear relation with GCA risk. Similar trends were found for tree nut and peanut intake, which were mostly nonsignificant.

For peanut butter intake, nonsignificant positive associations were found with the esophageal cancer subtypes and unclear associations with the gastric cancer subtypes. Statistical evidence for nonlinearity was only observed for the relation between total nut consumption and GNCA risk. Moreover, no significant interactions between total nut consumption and risk factors for esophageal and gastric cancer subtypes were identified. When excluding the first four years of follow-up to reduce the influence of potential reversed causation, conclusions remained the same for EAC, GCA, and GNCA risk. The associations between nut consumption and ESCC risk attenuated, but remained inverse.

Our results are partially in accordance with a recent publication from the prospective NIH-AARP Diet and Health Study in the US [12]. In this US cohort, nut consumption was also associated with a significantly reduced GNCA risk during a median follow-up period of 15.5 years, but no relation was found with ESCC risk. Unfortunately, they did not investigate different nut types separately. Moreover, they observed a significant inverse association between peanut butter consumption and GNCA risk, while we found no significant relations with peanut butter. This difference may be due to the higher mean (SD) peanut butter intake in the US cohort (3.0 (7.4) g/day) than in our study (1.3 (3.9) g/day). Another possible explanation is the higher number of cases in the US cohort and thus the higher statistical power. In addition, we observed that higher consumption of nuts and peanut butter was non-significantly associated with an increased EAC risk. The positive relation for nut intake is an unexpected finding, for which we do not have a clear explanation. This results is not consistent with the results from the NIH-AARP Diet and Health Study, because they found no association between nut intake and EAC risk [12]. No other studies investigated this association, and therefore further research regarding the relation between nut consumption and EAC risk is required.

Table 3 Multivariable-adjusted HRs (95% CIs) for risk of esophageal and gastric cancer subtypes according to nut intake after excluding the first four years of follow-up; Netherlands Cohort Study on diet and cancer (NLCS), 1986-2006

		Subcohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric non-cardia adenocarcinoma	
	Median[a]	Person time at risk (years)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)
Total nut consumption (g/day)										
0.0	0.0	16,829	44	1 (ref)	54	1 (ref)	53	1 (ref)	213	1 (ref)
0.1-<5	2.5	16,813	41	1.05 (0.66-1.66)[c]	62	1.23 (0.83-1.81)	57	1.05 (0.70-1.58)	152	0.75 (0.59-0.95)[c]
5-<10	8.2	5,991	14	0.95 (0.49-1.84)[d]	27	1.41 (0.85-2.34)	21	0.99 (0.56-1.76)	45	0.63 (0.44-0.90)
10+	19.6	8,673	11	0.46 (0.23-0.94)	38	1.19 (0.74-1.91)	32	0.91 (0.56-1.49)	75	0.64 (0.47-0.87)
P_{trend}				0.018		0.688		0.620		0.022
Continuous, per 5 g/day increment										
Overall		48,305	110	0.93 (0.82-1.06)	181	1.04 (0.99-1.10)	163	0.99 (0.93-1.05)	485	0.93 (0.88-0.99)
Men		22,111	61	0.97 (0.85-1.11)	141	1.03 (0.97-1.09)	133	1.00 (0.94-1.06)	316	0.94 (0.89-1.00)
Women		26,195	49	0.83 (0.65-1.05)	40	1.18 (1.05-1.33)	30	0.84 (0.61-1.15)	169	0.87 (0.73-1.03)
Tree nut consumption (g/day)										
0.0	0.0	34,093	88	1 (ref)	132	1 (ref)	121	1 (ref)	374	1 (ref)
0.1+	2.1	14,212	22	0.67 (0.41-1.09)	49	1.04 (0.74-1.48)	42	0.95 (0.64-1.40)	111	0.84 (0.66-1.06)
Continuous, per 5 g/day increment										
Overall		48,305	110	0.72 (0.45-1.13)	181	1.03 (0.85-1.26)	163	0.70 (0.42-1.17)	485	0.83 (0.67-1.02)
Men		22,111	61	0.52 (0.23-1.16)	141	0.99 (0.80-1.22)	133	0.77 (0.46-1.27)	316	0.92 (0.75-1.12)[d]
Women		26,195	49	0.85 (0.50-1.42)[d]	40	1.18 (0.92-1.51)[c]	30	0.24 (0.06-1.01)	169	0.49 (0.31-0.79)
Peanut consumption (g/day)										
0.0	0.0	19,515	48	1 (ref)	63	1 (ref)	61	1 (ref)	229	1 (ref)
0.1-<5	2.5	17,721	42	1.04 (0.67-1.63)[c]	65	1.14 (0.78-1.67)	57	0.95 (0.64-1.40)	157	0.79 (0.62-0.99)[c]
5-<10	8.5	4,443	12	1.06 (0.53-2.13)[d]	21	1.31 (0.75-2.29)	16	0.90 (0.49-1.64)	37	0.67 (0.45-0.99)
10+	21.4	6,626	8	0.46 (0.21-1.00)	32	1.19 (0.72-1.97)	29	1.01 (0.61-1.67)	62	0.73 (0.52-1.01)
P_{trend}				0.043		0.586		0.959		0.106

(continued)		Subcohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric non-cardia adenocarcinoma	
	Median[a]	Person time at risk (years)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)
Continuous, per 5 g/day increment										
Overall		48,305	110	0.94 (0.83-1.08)	181	1.04 (0.99-1.10)	163	1.00 (0.94-1.06)	485	0.94 (0.89-1.00)
Men		22,111	61	0.99 (0.87-1.12)	141	1.03 (0.97-1.09)	133	1.01 (0.95-1.07)	316	0.94 (0.88-1.00)
Women		26,195	49	0.80 (0.62-1.03)	40	1.18 (1.04-1.35)	30	0.86 (0.60-1.24)	169	0.91 (0.76-1.09)
Peanut butter consumption (g/day)										
0.0	0.0	34,906	82	1 (ref)	127	1 (ref)	120	1 (ref)	359	1 (ref)
0.1-<5	1.2	8,424	15	0.92 (0.53-1.63)	36	1.29 (0.87-1.92)[d]	32	1.15 (0.76-1.75)	85	1.09 (0.84-1.42)
5+	9.6	4,975	13	1.55 (0.82-2.93)	18	1.10 (0.65-1.87)	11	0.65 (0.34-1.25)	41	0.83 (0.58-1.18)
P_{trend}				0.184		0.599		0.252		0.319
Continuous, per 5 g/day increment										
Overall		48,305	110	1.29 (1.07-1.57)	181	1.14 (0.97-1.34)	163	0.88 (0.66-1.17)	485	0.92 (0.79-1.06)
Men		22,111	61	1.38 (1.09-1.74)[d]	141	1.18 (1.00-1.39)	133	0.98 (0.77-1.24)	316	0.91 (0.77-1.08)
Women		26,195	49	1.12 (0.74-1.69)	40	0.74 (0.37-1.44)	30	0.05 (0.00-1.05)	169	0.94 (0.69-1.27)

[a] Median in the subcohort

[b] Adjusted for age (years; continuous), sex, cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5/18.5-<25/25-<30/30+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of esophageal cancer (for esophageal cancer subtypes; no/yes), family history of gastric cancer (for gastric cancer subtypes; no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

[c] The proportional hazards assumption was violated for the exposure variable, and there was a statistically significant interaction with time

[d] The proportional hazards assumption was violated for the exposure variable, but there was no statistically significant interaction with time

Results from several case-control studies are inconclusive: for gastric adenocarcinoma risk, one case-control study found a significant positive trend with frequency of nut consumption [13], two observed significant inverse associations when comparing cases to population controls [15, 16], and one found no association with total nut consumption [14]. For ESCC, a significant inverse association was observed with peanut consumption frequency in one case-control study [17]. In another case-control study, no relation was seen between fruit and nut consumption combined and risk of aerodigestive tract cancer [18]. These contradicting results may be caused by the small sample sizes of the studies, and due the fact that these studies were vulnerable to selection and information biases, a problem intrinsic to all case-control studies.

Reversed causation may be another explanation for the inconsistent findings in the above described case-control studies. People with esophageal or gastric cancer may already suffer from symptoms related to their disease before diagnosis. These prediagnostic complaints may result in changes in diet and, consequently, in biased recall of previous normal dietary habits; this phenomenon has been described previously [29]. Asking participants about their diet long before the onset of symptoms would not solve this problem, since recall of remote diet is strongly influenced by current diet [30]. In general, cohort studies are less vulnerable to reversed causation than case-control studies because of the longitudinal design. In line with this reasoning, we observed in our study that the median nut consumption significantly differed between ESCC cases diagnosed after different follow-up durations. To investigate the effect of potential reversed causation on our results, we conducted sensitivity analyses in which we excluded the first four years of follow-up. This did not change the conclusions about the relation of nut and peanut butter consumption with EAC, GCA, and GNCA risk. The associations between nut consumption and ESCC risk attenuated, but remained inverse. This clearly underlines the importance of taking into account possible information bias due to reversed causation, especially in case-control studies.

Nuts are rich sources of vitamins (e.g. B6, B9, and E), minerals (e.g. selenium and magnesium), fiber, proteins, mono- and polyunsaturated fatty acids, phytosterols, and polyphenols (e.g. flavonoids (quercetin, genistein), stilbenes (resveratrol), and ellagic acid) [5, 6]. However, the nutrient composition varies between nut types. Peanuts, which are botanically legumes, contain comparable amounts of total fat, protein, and fiber as almonds, but higher amounts of saturated fatty acids, folate and phytosterols [31]. Walnuts contain less protein, fiber, and folate than peanuts, and more total fat, which is mainly due to its high poly-unsaturated fatty acid content [31]. Peanut butter contains the beneficial components of peanuts, although some additives are supplemented to enhance its quality, taste, and presentation [32]. The main proposed mechanisms by which nuts might conduct their cancer-chemopreventive effects relate to their antioxidant and anti-inflammatory effects [5, 6]. Other hypothesized mechanisms are the regulation of cell differentiation, proliferation and apoptosis, inhibition of tumor initiation, modulation of angiogenesis, induction of DNA damage repair and detoxifying metabolic enzymes, modification of hormonal mechanisms, and alteration of lipid profiles and cell metabolism [5, 6].

EAC mainly develops in the distal esophagus and GCA in the gastric cardia. Therefore, it might be difficult to determine whether a large tumor near the gastroesophageal junction has an esophageal or a gastric origin [33]. Consequently, misclassification of EAC and GCA tumors might occur. In the NLCS, information on cancer incidence was obtained from the Netherlands Cancer Registry. This registry combines pathology and clinical information to obtain high quality data regarding the topography and histology of tumors, which has been reported to be of high accuracy [34].

The number of esophageal cancer and GCA cases in our study was fairly limited, because of the relatively low incidence of these cancers in the Netherlands [35]. Nevertheless, the large size of the NLCS and the long follow-up of 20.3 years enabled us to analyze the esophageal and gastric cancer subtypes separately. Due to limited power, we could not perform the analyses for males and females separately, except for the continuous analyses. In the continuous analyses, the associations appeared to be stronger in females than in males. Therefore, we recommend to investigate sex-specific relations in future studies. Another strength is that we were able to investigate the effects of different types of nuts on the risk of esophageal and gastric cancer subtypes. Moreover, the prospective design and the high completeness of follow-up make selection and information bias unlikely.

Our results were adjusted for many possible confounders, but residual confounding by unmeasured factors still may occur. No data on *H. Pylori* infection was collected at baseline in 1986, and therefore we could not adjust for this factor. In a Dutch study published in 2013, the seroprevalence of *H. Pylori* was estimated to be 48% among Dutch blood donors born between 1935 and 1946 and 16% among those born between 1977 and 1987, indicating a birth-cohort effect [36]. Because the participants in our study were born between 1916 and 1932, we expect the prevalence in our study population to be higher than 48%. *H. Pylori* infection has been shown to increase GNCA risk, whereas it decreases EAC risk and possibly also GCA risk in low-risk settings [37, 38]. Therefore, *H. Pylori* infection may be a confounder if it is also associated with nut intake. If *H. Pylori* infection would be related to a reduced nut intake because of stomach complaints or decreased appetite, then the inverse association between nut intake and GNCA risk might be an overestimation. However, no evidence regarding this association is available. In addition, in recent years, several publications have hypothesized that statin use reduces esophageal and gastric cancer risk [39-41]. We recommend future studies to take these factors into account as well.

In conclusion, increased nut consumption was associated with a reduced risk of GNCA in this large prospective cohort study, and possibly with a reduced risk of ESCC. Peanut butter was not significantly related with gastric or esophageal cancer risk.

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Supplementary Table 1 Age- and sex-adjusted HRs (95% CIs) for risk of esophageal and gastric cancer subtypes according to nut intake; Netherlands Cohort Study on diet and cancer (NLCS), 1986-2006

		Subcohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric non-cardia adenocarcinoma	
	Median[a]	Person time at risk (years)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)
Total nut consumption (g/day)										
0.0	0.0	22,062	59	1 (ref)	60	1 (ref)	61	1 (ref)	252	1 (ref)
0.1-<5	2.5	21,821	42	0.73 (0.49-1.10)[c]	68	1.09 (0.76-1.55)	72	1.13 (0.79-1.61)	184	0.74 (0.60-0.92)[d]
5-<10	8.2	7,753	15	0.74 (0.41-1.31)[c]	29	1.20 (0.75-1.91)	22	0.88 (0.53-1.48)	51	0.57 (0.41-0.79)
10+	19.9	11,269	17	0.55 (0.31-0.96)	43	1.10 (0.72-1.66)	36	0.87 (0.57-1.34)	99	0.70 (0.54-0.90)[c]
P_{trend}				0.061		0.775		0.329		0.027
Continuous, per 5 g/day increment										
Overall		62,905	133	0.98 (0.89-1.08)	200	1.04 (1.00-1.09)	191	0.98 (0.92-1.04)	586	0.95 (0.91-1.00)[c]
Men		29,250	76	1.00 (0.90-1.10)	157	1.03 (0.98-1.08)	158	0.99 (0.93-1.05)	390	0.96 (0.92-1.01)
Women		33,655	57	0.89 (0.72-1.10)	43	1.13 (1.03-1.23)	33	0.90 (0.72-1.12)	196	0.89 (0.77-1.03)
Tree nut consumption (g/day)										
0.0	0.0	44,547	110	1 (ref)	145	1 (ref)	142	1 (ref)	455	1 (ref)
0.1+	2.1	18,358	23	0.53 (0.33-0.83)	55	0.95 (0.69-1.31)	49	0.88 (0.63-1.23)	131	0.74 (0.60-0.91)
Continuous, per 5 g/day increment										
Overall		62,905	133	0.60 (0.35-1.04)	200	1.04 (0.90-1.20)	191	0.69 (0.43-1.10)	586	0.76 (0.61-0.95)
Men		29,250	76	0.45 (0.20-1.00)	157	1.04 (0.89-1.22)	158	0.74 (0.47-1.18)	390	0.85 (0.68-1.06)
Women		33,655	57	0.77 (0.40-1.48)	43	1.04 (0.73-1.47)	33	0.34 (0.11-1.03)	196	0.50 (0.32-0.79)
Peanut consumption (g/day)										
0.0	0.0	25,552	63	1 (ref)	71	1 (ref)	70	1 (ref)	269	1 (ref)
0.1-<5	2.5	22,960	43	0.78 (0.52-1.17)[c]	70	1.02 (0.72-1.44)	71	1.03 (0.73-1.46)	192	0.80 (0.65-0.99)[d]
5-<10	8.5	5,762	13	0.92 (0.49-1.72)[d]	23	1.17 (0.71-1.94)	17	0.86 (0.49-1.50)	43	0.68 (0.48-0.96)
10+	21.4	8,630	14	0.63 (0.34-1.16)	36	1.13 (0.73-1.73)	33	1.00 (0.65-1.55)	82	0.80 (0.61-1.06)[c]
P_{trend}				0.224		0.583		0.884		0.198

(continued)		Subcohort	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric non-cardia adenocarcinoma	
	Median[a]	Person time at risk (years)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)	No. of cases	HR[b] (95% CI)
Continuous, per 5 g/day increment										
Overall		62,905	133	1.00 (0.91-1.10)	200	1.04 (1.00-1.09)	191	1.00 (0.94-1.06)	586	0.97 (0.92-1.01)[c]
Men		29,250	76	1.01 (0.93-1.11)	157	1.03 (0.98-1.09)	158	1.00 (0.94-1.06)	390	0.97 (0.93-1.02)[c]
Women		33,655	57	0.90 (0.72-1.12)	43	1.16 (1.05-1.30)	33	0.92 (0.72-1.18)	196	0.93 (0.80-1.08)
Peanut butter consumption (g/day)										
0.0	0.0	45,489	98	1 (ref)	140	1 (ref)	139	1 (ref)	433	1 (ref)
0.1-<5	1.2	10,970	20	0.89 (0.54-1.46)	37	1.14 (0.78-1.66)[c]	37	1.13 (0.77-1.66)	101	1.03 (0.81-1.30)
5+	9.6	6,446	15	1.12 (0.64-1.96)	23	1.13 (0.71-1.79)	15	0.73 (0.42-1.27)	52	0.87 (0.64-1.19)
P_{trend}				0.655		0.464		0.350		0.451
Continuous, per 5 g/day increment										
Overall		62,905	133	1.10 (0.91-1.32)	200	1.11 (0.97-1.26)	191	0.88 (0.69-1.11)	586	0.93 (0.81-1.05)
Men		29,250	76	1.13 (0.93-1.36)	157	1.14 (1.01-1.29)	158	0.94 (0.76-1.15)	390	0.92 (0.80-1.06)
Women		33,655	57	1.00 (0.67-1.49)	43	0.60 (0.27-1.31)	33	0.11 (0.01-0.83)	196	0.93 (0.70-1.24)

[a] Median in the subcohort

[b] Age- and sex-adjusted

[c] The proportional hazards assumption was violated for the exposure variable, and there was a statistically significant interaction with time

[d] The proportional hazards assumption was violated for the exposure variable, but there was no statistically significant interaction with time

Supplementary Table 2 Multivariable-adjusted[a] interactions between total nut intake categories (0, 0.1-<5, and 5+ g/day) and potential risk factors for esophageal and gastric cancer subtypes, the Netherlands Cohort Study, 1986-2006

	Esophageal squamous cell carcinoma	Esophageal adenocarcinoma	Gastric cardia adenocarcinoma	Gastric non-cardia adenocarcinoma
	$P_{interaction}$	$P_{interaction}$	$P_{interaction}$	$P_{interaction}$
Sex (male/female)	0.599	0.206	0.808	0.573
Smoking status (never/former/current)	0.738	0.240	0.692	0.779
BMI (18.5-<25.0/≥25 kg/m^2)	0.358	0.133	0.054	0.784
Alcohol consumption (0/0.1-<15/≥15 g/day)	0.748	0.337	0.522	0.514
Educational level (low/medium/high)	0.347	0.043	0.321	0.376

[a] Adjusted for age (years; continuous), sex, cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5/18.5-<25/25-<30/30+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of esophageal cancer (for esophageal cancer subtypes; no/yes), family history of gastric cancer (for gastric cancer subtypes; no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

Supplementary Table 3 HRs (95% CIs) for esophageal adenocarcinoma according to total nut intake in strata of educational level, in multivariable-adjusted analyses[a], the Netherlands Cohort Study, 1986-2006

	Esophageal adenocarcinoma		
	Total nut consumption (g/day)		
	0	0.1-<5	5+
Educational level			
Low			
Cases/person time at risk (years)	35/12,626	35/10,618	33/7,359
HR	1 (ref)	1.17	1.44
95% CI	-	0.70-1.94	0.85-2.44
P_{trend}			0.232
Medium			
Cases/person time at risk (years)	16/7,129	16/7,959	31/7,744
HR	1 (ref)	1.00	1.84
95% CI	-	0.47-2.11	0.85-3.98
P_{trend}			0.071
High			
Cases/person time at risk (years)	9/2,306	17/3,244	8/3,920
HR	1 (ref)	1.93	0.42
95% CI	-	0.72-5.17	0.14-1.26
P_{trend}			0.013
$P_{interaction}$			0.043

[a] Adjusted for age (years; continuous), sex, cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5/18.5-<25/25-<30/30+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), family history of esophageal cancer (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

Supplementary Table 4 Median total nut intake at baseline of esophageal and gastric cancer cases diagnosed over the follow-up time, the Netherlands Cohort Study, 1986-2006

	Esophageal squamous cell carcinoma		Esophageal adenocarcinoma		Gastric cardia adenocarcinoma		Gastric non-cardia adenocarcinoma	
	n	Median intake (g/day) (IQR)	n	Median intake (g/day) (IQR)	n	Median intake (g/day) (IQR)	n	Median intake (g/day) (IQR)
Time between baseline and cancer diagnosis (years)								
0-<4	23	0.0 (0.0-14.2)	19	2.1 (0.0-12.8)	28	1.9 (0.0-4.1)	101	1.8 (0.0-8.5)
4-<8	30	0.0 (0.0-4.3)	39	2.8 (0.0-7.0)	33	2.0 (0.0-4.9)	130	1.6 (0.0-4.5)
8-<12	27	1.0 (0.0-4.3)	34	2.0 (0.0-8.5)	56	2.0 (0.8-8.5)	128	1.8 (0.0-6.7)
12-<16	25	1.0 (0.0-3.6)	46	4.3 (0.0-10.2)	40	2.0 (0.0-9.9)	115	0.0 (0.0-3.6)
16+	28	4.6 (2.0-8.5)	62	3.5 (0.8-9.4)	34	2.4 (0.0-8.5)	112	1.0 (0.0-8.5)
P		0.019		0.636		0.832		0.077

Chapter 3

Nut and peanut butter intake and the risk of colorectal cancer and its anatomical and molecular subtypes: The Netherlands Cohort Study

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Abstract

Introduction: Nut intake has been associated with reduced total cancer-related mortality, but evidence for colorectal cancer (CRC) risk is inconclusive. We investigated the associations between nut and peanut butter intake and anatomical CRC subtypes. To account for molecular heterogeneity, associations between nut and peanut butter intake and colorectal tumors harboring *APC*, *KRAS*, or *BRAF* mutations, p53 overexpression, or microsatellite instability were examined in secondary analyses.

Methods: In the Netherlands Cohort Study (n=120 852), lifestyle habits were measured with a questionnaire in 1986. After 20.3 years of follow-up, 3567 CRC cases were included in case-cohort analyses. For the analyses of molecular CRC subtypes, 574 CRC cases were included after 7.3 years of follow-up.

Results: In categorical analyses, total nut intake was not significantly associated with CRC (HR (95%CI) for 10+ g/day versus nonconsumers = 0.94 (0.78-1.15) in men and 0.96 (0.75-1.22) in women). In restricted cubic spline analyses, significant nonlinear inverse associations with rectal cancer were observed for total nut, peanut, and peanut butter intake in women, and borderline significant nonlinear inverse associations for total nut and peanut intake in men. Regarding the molecular CRC subtypes, peanut butter intake was significantly associated with an increased risk of colorectal tumors that did not develop through the serrated neoplasia pathway in men (HR (95%CI) per 5 g/day increment = 1.22 (1.07-1.38)).

Conclusion: Nut and peanut butter intake are nonlinearly inversely associated with rectal cancer risk in women. In men, nut intake is borderline significantly nonlinearly associated with a reduced rectal cancer risk. Peanut butter is associated with an increased risk of colorectal tumors that do not develop through the serrated neoplasia pathway in men.

Introduction

In 2018, colorectal cancer (CRC) was estimated to be the third most common cancer worldwide, accounting for 10.2% of all new cancer cases, and the second most frequent cause of cancer-related mortality (1). The global incidence of CRC has been estimated to rise to more than 2.2 million new cases and 1.1 million deaths by 2030, which is partly caused by changes in the prevalence of lifestyle factors with more people adopting a more Western diet and lifestyle (2).

Nuts represent a food group that has been studied for its potential cancer-chemopreventive activities, because recent studies have shown that nut consumption is inversely associated with total cancer-related mortality (3). Nuts contain numerous bioactive compounds like vitamins B6 and E, folate, selenium, fiber, mono- and polyunsaturated fatty acids and polyphenols. The nutritional composition differs per nut subtype (4,5). Possible mechanisms by which nuts might reduce CRC risk relate to their antioxidant activities and anti-inflammatory effects (4,6,7). Bioactive compounds in nuts might contribute to normal cell differentiation and DNA repair mechanisms, reduced tumor initiation, promotion, and angiogenesis, and induced apoptosis (6,7). Fiber in nuts increase fecal volume, dilute fecal carcinogens, and decrease the intestinal transit time, thereby reducing the contact between carcinogens and the intestinal lining (7,8). However, the exact working mechanisms and the differential effects of nut subtypes have yet to be elucidated.

To our knowledge, eight prospective cohort studies (9-16) and eight case-control studies (17-24) have investigated the relation between nut and peanut butter intake and CRC risk. Two cohort studies found no significant associations between nut intake and CRC risk in both sexes (10,11). In men, five cohort studies observed no significant associations (9,11,13-15). In women, one cohort study found significant inverse associations of nut intake with colon and distal colon cancer, but not with colorectal, proximal colon, and rectal cancer (11). Another cohort study in women observed significant inverse relations between peanut intake and CRC risk (13), and a third observed significant inverse relations between total nut intake and colon cancer risk (16). Conversely, two cohort studies found no relations in women (12,14,15). No cohort studies were performed on peanut butter. Results from the case-control studies were contradictive as well (17-24).

The contradicting results might partly be explained by the fact that CRC is a heterogeneous disease with several molecular subtypes, each characterized by certain (epi)genetic abnormalities (25). There are minimally two hypothesized pathways to CRC development. The traditional adenoma-carcinoma pathway accounts for 60-90% of all sporadic CRC tumors, which are more often observed in men and the distal colon, and is characterized by chromosomal instability (25). Mutations in the Adenomatous Polyposis Coli (*APC*) tumor suppressor gene often occur early in this pathway, while mutations in the Kirsten ras (*KRAS*) proto-oncogene and in *TP53* are common later events (25). The serrated neoplasia pathway accounts for 10-30% of all sporadic CRC tumors, which are more frequently observed in

women and the proximal colon, and is characterized by microsatellite instability (MSI) (25). Mutations in the *B-RAF* proto-oncogene serine/threonine kinase (*BRAF*) are early events in this pathway (25).

Diet and lifestyle may play important roles in causing mutations and epigenetic alterations, and can influence tumor growth in tissues that already underwent (epi)genetic changes (25). Therefore, they might be associated with molecular characteristics in CRC. If such associations are observed, this may point to distinct underlying molecular pathways linking diet and lifestyle to cancer and to distinct etiologies of molecular CRC subtypes. This will strengthen the evidence-base needed for prevention.

In previous studies, several nut components have been found to be associated with the molecular CRC subtypes (25). High intake of polyunsaturated fat, especially linoleic acid, has been positively associated with mutated *KRAS* colorectal tumors (26). In contrast, (marine) omega-3 polyunsaturated fatty acid intake was found to be associated with a lower risk of MSI-high tumors, but not MS-stable (MSS) tumors (27). Regarding folate intake, a positive associations has been found with mutated *BRAF* colorectal tumors (28), but high folate consumption has also been associated with a reduced risk of mutated *KRAS* rectal tumors in men (29) and wild-type *APC* colon tumors, while being positively associated with mutated *APC* colon tumors in men (30). However, there is no prospective evidence for associations between nut or peanut butter intake and molecular CRC subtypes.

As primary aim, we investigated the associations between total nut, tree nut, peanut, and peanut butter intake and the risk of anatomical CRC subtypes in men and women, using data from the Netherlands Cohort Study (NLCS) with 20.3 years of follow-up. As secondary aim, we performed exploratory and hypothesis-generating analyses to examine the associations between nut intake and molecular CRC subtypes, characterized by mutational status of *APC*, *KRAS*, *BRAF*, and MSI and p53-status, using an existing database of molecular tumor characteristics of CRC cases who were diagnosed during the first 7.3 years of follow-up in the NLCS.

Materials and methods

The NLCS is a population-based prospective cohort study in the Netherlands, which started on 17 September 1986 (31). In total, 120 852 men and women aged 55-69 years at baseline were included. A case-cohort approach was applied to improve the efficiency of the follow-up and data processing. A subcohort of 5000 participants was randomly sampled from the entire cohort directly after baseline. Cases were obtained from the entire cohort, whereas person-time was estimated in the subcohort as an estimate of the follow-up time in the total cohort.

In September 1986, participants filled in a mailed, self-administered baseline questionnaire on diet, lifestyle habits, and other cancer risk factors. This questionnaire included a

validated semi-quantitative 150-item food frequency questionnaire (FFQ) that covered information on dietary habits in the year preceding baseline (32). By filling in and returning the baseline questionnaire, participants agreed to participate in the NLCS. The NLCS was conducted in agreement with the Declaration of Helsinki. The institutional review boards of the Netherlands Organization for Applied Scientific Research TNO (Zeist) and Maastricht University (Maastricht) approved the NLCS.

The entire cohort was followed-up for cancer incidence until 31 December 2006 by record linkage to the Dutch Pathology Registry (PALGA) and Dutch Cancer Registry (33), providing a coverage of approximately 96% (34). The subcohort was followed-up biennially for data on vital status, which was estimated to be 100% complete.

Study population

In our primary analyses, the study population consisted of subcohort members and incident microscopically confirmed CRC cases diagnosed during 20.3 years (baseline until December 2006) of follow-up. Participants were excluded if they reported prevalent cancer (excluding skin cancer) at baseline, if they had inconsistent or incomplete dietary data, or if they had missing data on potential confounding variables.

For the anatomical analyses, 3567 CRC cases (ICD-O-3 codes: C18-20) remained eligible after applying the in- and exclusion criteria, including 2483 colon cancer cases (C18) and 752 rectal cancer cases (C20) (Supplementary Figure 1). Of the colon cancer cases, 1292 were categorized as proximal (C18.0-18.4) and 1120 as distal colon cancer cases (C18.5-18.7). Malignant tumors of the rectosigmoid junction (C19) were only included in the overall CRC analyses, because of the higher risk of misclassification.

For the molecular analyses, paraffin-embedded tumor tissue samples of 732 CRC cases detected during 7.3 years of follow-up (September 1986-December 1993) were collected and analyzed as described in (35). Because of incomplete nationwide coverage by PALGA in the earlier years, the first 2.3 years of follow-up were excluded. The number of CRC cases per molecular subtype are presented in Supplementary Figure 2.

***APC*, *KRAS*, and *BRAF* mutations**

The methods of DNA isolation, PCR, and sequencing have been described elsewhere (35-37). For the *APC* gene, tumor material was analyzed for mutations in the mutation cluster region spanning codons 1286-1520 of exon 15. For the *KRAS* oncogene, codons 12-13 of exon 1 were analyzed, and for the *BRAF* gene the V600E *BRAF* mutation in exon 15. For the *APC* and *KRAS* genes, a nested PCR method was performed, followed by direct sequencing of purified segments (35,36). For the *BRAF* gene, a semi-nested PCR and restriction fragment length polymorphism (RFLP) analyses were performed (37).

Microsatellite instability

MSI was determined by a pentaplex PCR using the MSI markers BAT-26, BAT-25, NR-21, NR-22 and NR-24 (38). Tumors were classified as MS-unstable (MSI) if ≥2 markers showed

instability and as MSS if ≤1 of the markers showed instability.

***TP53* expression**

Immunohistochemical staining of p53 was performed using the avidin–biotin–peroxidase complex method, with the DO-7 mouse monoclonal antibody (DAKO A/S, Denmark) (39). Immunostained slides and negative controls were evaluated semi-quantitatively and independently by two observers without knowledge of clinical parameters. Cases were positive for *TP53* overexpression if ≥20% of the tumor cell nuclei showed positive staining with the antibody (39).

Exposure measurement

The baseline questionnaire measured lifestyle habits, dietary intakes, and other cancer risk factors. In the 150-item FFQ, three items covered intake of 'peanuts', 'other, mixed nuts' (tree nuts), and 'peanut butter' in the preceding year. Participants filled in the intake frequencies and number of portion sizes they consumed per intake. Intake frequencies could range from 'never or <1x/month' to '6-7x/week'. Assumed standard portion sizes were 28 grams for peanuts and tree nuts and 15 grams per slice of bread for peanut butter (32). Mean daily intakes were calculated by multiplying intake frequencies and portion sizes. Total nut intake was the sum of tree nut and peanut intake. Peanut butter intake was not included in total nut intake, because its nutrient composition differs from that of nuts (5). NLCS personnel was blinded to the case/subcohort status of participants during the entry, coding, and interpretation of the questionnaire data.

Statistical analysis

Age- and multivariable-adjusted Cox regression analyses were performed for men and women separately. In our primary analyses, we investigated the relation between total nut intake and CRC risk. The analyses were also performed for tree nut, peanut, and peanut butter intake and for colon, proximal colon, distal colon, and rectal cancer. Person-years in the subcohort were calculated from baseline (September 1986) until CRC diagnosis, loss to follow-up, death, migration, or end of follow up (December 2006), whichever came first.

The proportional hazards assumption was tested using scaled Schoenfeld residuals (40) and by visually inspecting log-minus-log survival plots. Because a potential violation was observed for age, a time-varying covariate was included for age. Additional variance introduced by sampling the subcohort from the full cohort was taken into account by using the robust Huber-White sandwich estimator (41).

Total nut, tree nut, peanut, and peanut butter intakes were analyzed on a categorical and continuous scale. Total nut and peanut intake were categorized into 0, 0.1-<5, 5-<10, and 10+ g/day, and tree nut and peanut butter intake into 0, 0.1-<5, and 5+ g/day because of the smaller number of participants in the higher intake categories. Nonconsumers formed the reference category. Linear trends were evaluated by assigning median intakes in the subcohort to the intake categories, fitting these as continuous variables in regression models, and performing Wald tests. In continuous analyses, increments of 5 g/day were

analyzed.

Predefined confounders included age (years; continuous), cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5/18.5-<25/25-<30/30+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (primary or lower vocational education (low)/ secondary or medium vocational education (medium)/higher vocational education or university (high)), family history of CRC (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day). Cigarette smoking frequency and duration were centered to reduce multicollinearity between the smoking variables (42). Potential confounders included height, consumption of fruits, vegetables, whole grains, red meat, processed meat, fish, dairy products, cheese, and coffee, nutritional supplement use, postmenopausal hormone replacement therapy (in women), long-term use (>6 months) of nonsteroidal anti-inflammatory drugs (including aspirin), history of chronic bowel disease, and history of diabetes. Predefined confounders were included in the multivariable-adjusted models irrespective of their effect on the HRs. None of the potential confounders changed the HRs with ≥10% when using a backward selection procedure, so only the predefined confounders were included in the final model.

In nutritional epidemiologic research, linear relations are uncommon because the capacity for absorbing, transporting, and metabolizing dietary factors is often limited (43). Therefore, we tested for nonlinearity using restricted cubic spline analyses with three fixed knots at 0, 5, and 10 g/day. In sensitivity analyses, this model was compared to models with different knot positions or additional knots using the Akaike Information Criterion score (44).

Heterogeneity in the associations between nut intake and the risk of anatomical CRC subtypes was tested using a competing risk procedure (28), which estimates standard errors using a bootstrapping method (1000 replications) specifically designed for the case-cohort design (45).

Stratified analyses were performed to investigate associations between total nut intake and colon and rectal cancer risk across levels of BMI (18.5-<25/25+ kg/m^2), nonoccupational physical activity (≤30/30-≤60/60-≤90/>90 min/day), cigarette smoking status (never/former/current), alcohol consumption (0/0.1-<5/≥5 g/day), educational level (low/medium/high), and family history of CRC (no/yes) to exploratively investigate potential effect modification. Participants with a BMI <18.5 kg/m^2 were excluded from the interaction analyses, because of the low number of participants in this category. The two highest intake categories were merged to increase statistical power. Interactions were tested by including cross-product terms in the Cox regression models and performing Wald tests.

In sensitivity analyses, we mutually adjusted tree nut, peanut, and peanut butter intakes and we additionally adjusted for the alternate Mediterranean diet (aMED) score excluding alcohol and nuts (46). Potential reversed causation was examined by comparing the median total nut intake of cases diagnosed during the first two years of follow-up to the

median intakes of cases diagnosed later in time, and by excluding the first two years of follow-up. Moreover, we restricted the peanut butter analyses to participants with a self-reported constant peanut butter intake in the five years before baseline. This information was obtained from the FFQ, in which participants were asked whether they used more, less, or just as much peanut butter five years before baseline as they did at baseline. This information was collected for peanut butter intake, but unfortunately not for tree nuts and peanuts.

Two-sided *P*-values <0.05 were considered statistically significant. All analyses were performed in Stata 14 software (StataCorp.2015. College Station, TX).

Molecular subtypes

In the molecular analyses, we investigated the relation between nut and peanut butter intake and the following molecular CRC endpoints: the presence of truncating *APC* mutations (no/yes); activating *KRAS* mutations (no/yes); p53 overexpression (no/yes); *BRAF* mutations (no/yes); MSI (no/yes). The relation between nut intake and CRC developed through the traditional adenoma-carcinoma pathway was investigated by examining truncating *APC* mutations and/or activating *KRAS* mutations and/or p53 overexpression as combined endpoint. *BRAF* mutations and/or MSI were combined as marker of the serrated neoplasia pathway. Unfortunately, the molecular analyses could not be performed for colon or rectal cancer separately, because of the small number of cases and the skewed distribution of nut intake. We did not combine men and women, because the confounder patterns and the relation between nut intake and cancer risk differed between the sexes in previous studies (11,19).

In the molecular analyses, the same statistical approach was used as in the primary analyses. However, the follow-up period ran until December 1993 and excluded the first 2.3 years. Also, nut intake categories were merged and alcohol intake and BMI were included as continuous covariates because of the lower case numbers. There were no violations of the PH assumption, so no time-varying covariates were included in the models. Moreover, the *P*-values of the trend tests and continuous analyses were adjusted for multiple testing using the method of Benjamini-Hochberg (47), correcting for 44 tests per sex in the analyses of the individual molecular subtypes (Tables 3 and 4) and for 16 tests per sex in the analyses of the traditional adenoma-carcinoma and serrated neoplasia pathways (Table 5). This correction was only done in the analyses of the molecular subtypes because of their explorative nature, the limited number of cases, and the resulting less stable estimates.

Results

In Table 1, baseline characteristics stratified by sex are presented. In men, the mean (SD) total nut intake was 7.9 (13.7) g/day in the subcohort and 7.9 (13.2) g/day in CRC cases. In women, these intakes were 4.4 (8.5) and 4.3 (8.5) g/day, respectively. Tree nut, peanut, and peanut butter intakes were comparable in CRC cases and subcohort members. Female

rectal cancer cases had lower mean intakes of total nuts, tree nuts, and peanuts compared to subcohort members.

On average, CRC cases were somewhat older than subcohort members, more often ever smokers, and higher educated, more often reported a positive CRC family history, and reported higher alcohol intakes (Table 1). Male cases were on average heavier than subcohort members and more nonoccupationally physically active. Female cases were less nonoccupationally physically active than subcohort members.

Multivariable-adjusted HRs for the associations between nut and peanut butter intake and the risk of CRC and its anatomical subtypes in men and women are presented in Table 2. Age-adjusted results can be found in Supplementary Tables 1 and 2, for men and women respectively. Total nut intake was not associated with CRC risk in men or women in categorical and continuous multivariable-adjusted analyses. Compared with nonconsumers, the HR (95%CI) for 10+ g total nuts/day was 0.94 (0.78-1.15) (*P*-trend = 0.494) in men and 0.96 (0.75-1.22) (*P*-trend of linear test over all intake categories = 0.458) in women. No associations between tree nut, peanut, and peanut butter intake and CRC risk were observed in both sexes. Nut and peanut butter intake were also not associated with colon, proximal colon, and distal colon cancer in both sexes, except for a significant positive association between 0.1-<5 g total nut intake/day and distal colon cancer risk in women (HR (95%CI) = 1.31 (1.02-1.67)). In men, no significant associations were observed between nut and peanut butter intake and rectal cancer, except for a significant inverse association for 5-<10 g peanut intake/day versus nonconsumers (HR (95%CI) = 0.61 (0.40-0.92)). In women, also no significant associations were seen between nut and peanut butter intake and rectal cancer risk, except for a significant inverse association for 5-<10 g total nut intake/day versus nonconsumers (HR (95%CI) = 0.42 (0.23-0.76)).

The tests for heterogeneity across the anatomical CRC subtypes showed significant heterogeneity between overall colon and rectal cancer in women (*P*-heterogeneity = 0.029), but not in men (*P*-heterogeneity = 0.263). No significant heterogeneity was found between proximal and distal colon cancer in both sexes.

Restricted cubic spline curves for colon and rectal cancer according to nut and peanut butter intake are presented in Figure 1. For colon cancer, no associations were seen with nut or peanut butter intake in both sexes, and no statistical evidence for nonlinearity. For rectal cancer, the exposure response-curves showed significant inverse associations with total nut, peanut, and peanut butter intake in women, with a clear leveling-off of the exposure-response curves at intake of >7.5 g/day. The nonlinearity tests were significant for total nut and peanut intake in relation to rectal cancer in women (*P*-nonlinearity = 0.011 and 0.023, respectively), but not for tree nut or peanut butter intake. In men, the inverse associations between total nut and peanut intake and rectal cancer risk were borderline significant and no evidence for nonlinearity was found. Based on the AIC score, the model fit of the restricted cubic spline model with three fixed knots at 0, 5, and 10 g nut intake/day did not improve when using additional knots or different knot positions (data not shown).

Table 1. Baseline characteristics (mean (SD) or %) of subcohort members and colorectal cancer cases; NLCS, 1986-2006

	Men				Women			
	Subcohort	Colorectal cancer cases	Colon cancer cases	Rectal cancer cases	Subcohort	Colorectal cancer cases	Colon cancer cases	Rectal cancer cases
N	1834	1993	1296	475	1886	1574	1187	277
Age (years)	61.2 (4.2)	61.5 (4.1)	61.7 (4.2)	61.1 (4.0)	61.4 (4.2)	61.9 (4.1)	61.9 (4.1)	61.8 (4.0)
Body Mass Index (kg/m^2)	24.9 (2.6)	25.2 (2.6)	25.2 (2.7)	25.1 (2.5)	25.0 (3.5)	25.0 (3.5)	25.0 (3.5)	25.0 (3.5)
Ever cigarette smoker (%)	86.4	87.2	86.7	88.2	41.1	41.9	40.5	45.1
University or higher vocational education (%)	20.3	21.3	24.2	16.4	9.5	9.8	9.8	7.2
Nonoccupational physical activity (min/day)	81.0 (67.4)	82.5 (67.6)	82.7 (66.5)	85.5 (73.0)	65.5 (50.6)	62.9 (51.9)	62.7 (51.4)	63.7 (57.5)
Family history of colorectal cancer (%)	5.6	9.1	9.5	7.0	6.0	10.3	10.5	9.8
Daily energy intake (kcal)	2167 (499)	2150 (484)	2132 (488)	2200 (471)	1684 (389)	1681 (375)	1677 (382)	1690 (335)
Total nut intake (g/day)	7.9 (13.7)	7.9 (13.2)	7.8 (12.2)	8.0 (14.7)	4.4 (8.5)	4.3 (8.5)	4.5 (8.7)	3.6 (7.6)
Tree nut intake (g/day)	1.0 (3.4)	1.0 (3.4)	1.1 (3.5)	1.0 (3.4)	1.1 (4.0)	1.0 (3.1)	1.0 (3.0)	0.9 (3.4)
Peanut intake (g/day)	6.9 (13.0)	6.9 (12.4)	6.6 (11.3)	7.0 (13.9)	3.3 (7.0)	3.3 (7.2)	3.5 (7.4)	2.7 (6.2)
Peanut butter intake (g/day)	1.4 (4.2)	1.6 (4.2)	1.5 (4.1)	1.4 (3.7)	1.2 (3.6)	1.0 (3.5)	1.1 (3.8)	0.8 (2.4)
Alcohol intake (g/day)	15.1 (17.1)	16.3 (17.2)	15.6 (16.5)	17.6 (18.6)	6.0 (9.5)	6.3 (10.5)	6.2 (10.2)	6.5 (10.9)

In the analyses stratified by potential effect modifiers, we observed a significant interaction between total nut intake and cigarette smoking status in relation to colon cancer in women (*P*-interaction = 0.015; Supplementary Table 3): we observed no association between total nut intake and colon cancer risk in never and former smokers, and a significant positive association in current smokers. For rectal cancer (Supplementary Table 4), significant inverse trends with total nut intake were seen in men and women with this categorization (HR (95%CI) for 5+ g/day versus nonconsumers = (0.78 (0.60-1.03), *P*-trend of linear test over all intake categories = 0.043, and 0.58 (0.39-0.87), *P*-trend = 0.005, respectively). A significant interaction by BMI was found in men (*P*-interaction = 0.002), with a significant inverse association in normal weight men and a significant positive association in overweight men for the second nut intake category.

In sensitivity analyses, mutually adjusting tree nut, peanut, and peanut butter intake did not importantly change the results for the anatomical CRC subtypes, and neither did additionally adjusting for the aMED score excluding alcohol and nuts. When comparing the median total nut intake at baseline of colon and rectal cancer cases diagnosed over the follow-up period, we observed no increasing or decreasing trends (Supplementary Table 5). However, the Kruskal-Wallis tests was significant for total nut intake in male rectal cancer cases (*P* = 0.013). Excluding the first two years of follow-up or restricting the analyses of peanut butter to participants with a constant peanut butter intake in the five years before baseline did not change the results essentially.

Molecular subtypes

Baseline characteristics of subcohort members and cases per molecular CRC subtype are presented in Supplementary Table 6. Multivariable-adjusted HRs (95%CI) for the relation between nut and peanut butter intake and the risk of molecular CRC subtypes can be found in Tables 3 and 4. For total nut intake, no associations were observed in both sexes, except for significant inverse associations with total CRC and wild-type *KRAS* tumors in men (HR (95%CI) for 10+ g/day versus nonconsumers = 0.65 (0.45-0.95), *P*-trend of linear test over all intake categories = 0.060, and 0.54 (0.34-0.87), *P*-trend = 0.042, respectively). After FDR correction, the *P*-trend for wild-type *KRAS* tumors became nonsignificant. For tree nut intake, no associations with the molecular subtypes were observed in both sexes in the categorical analyses. In continuous analyses, the HR (95%CI) per 5 g tree nuts/day increment was 0.52 (0.28-0.95) for MSI tumors in women. This inconsistent finding between the categorical and continuous analyses is probably due to chance. Peanut intake was not associated with the molecular CRC subtypes in both sexes. For peanut butter intake, significant positive associations were found in wild-type *KRAS* tumors and tumors with p53 overexpression in men (HR (95%CI) for 5+ g/day versus nonconsumers = 1.55 (1.00-2.40), *P*-trend = 0.047, and 1.59 (1.01-2.52), *P*-trend = 0.045, respectively). After FDR correction, these *P*-trends became nonsignificant. In continuous analyses, significant positive associations with peanut butter were also seen in male cases with total CRC, with wild-type *KRAS* tumors, tumors with p53 overexpression, wild-type *BRAF* tumors, and MSS tumors (HR (95%CI) per 5 g/day increment = 1.14 (1.02-1.29), 1.15 (1.01-1.32), 1.21 (1.06-1.38), 1.20 (1.06-1.35), and 1.17 (1.03-1.34), respectively). However, the FDR-adjusted *P*-values were not significant for

Table 2. Multivariable-adjusted HRs and 95%CI for colorectal cancer and its anatomical subtypes in men and women, according to nut and peanut butter consumption; NLCS, 1986-2006

			Colorectal cancer		Colon cancer		Proximal colon cancer		Distal colon cancer		Rectal cancer	
	Median intake[a]	Person-years	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]
Men												
Total nut intake (g/day)												
0	0.0	8605	614	1.00 (reference)	397	1.00 (reference)	173	1.00 (reference)	218	1.00 (reference)	155	1.00 (reference)
0.1-<5	2.5	9509	668	1.00 (0.84-1.19)	426	0.99 (0.81-1.20)	196	1.06 (0.83-1.37)	213	0.87 (0.68-1.10)	168	1.02 (0.78-1.32)
5-<10	8.5	3896	252	0.93 (0.74-1.17)	170	0.96 (0.74-1.24)	78	1.04 (0.74-1.45)	82	0.80 (0.59-1.11)	48	0.72 (0.49-1.04)
10+	21.4	6936	459	0.94 (0.78-1.15)	303	0.98 (0.78-1.22)	142	1.11 (0.84-1.47)	155	0.87 (0.66-1.14)	104	0.82 (0.60-1.11)
P-trend				0.494		0.849		0.541		0.481		0.123
Continuous, per 5 g/day increment				0.99 (0.97-1.02)		0.99 (0.96-1.02)		1.01 (0.97-1.04)		0.98 (0.95-1.02)		0.99 (0.95-1.03)
Tree nuts (g/day)												
0	0.0	20 837	1453	1.00 (reference)	935	1.00 (reference)	421	1.00 (reference)	488	1.00 (reference)	346	1.00 (reference)
0.1-<5	1.6	6615	439	0.97 (0.82-1.14)	289	0.97 (0.80-1.17)	130	0.98 (0.77-1.25)	147	0.92 (0.73-1.16)	109	1.06 (0.82-1.36)
5+	8.9	1494	101	0.97 (0.71-1.32)	72	1.06 (0.76-1.49)	38	1.29 (0.86-1.95)	33	0.91 (0.59-1.41)	20	0.81 (0.48-1.35)
P-trend				0.774		0.791		0.253		0.593		0.485
Continuous, per 5 g/day increment				0.98 (0.89-1.07)		0.99 (0.90-1.09)		1.04 (0.93-1.15)		0.95 (0.83-1.09)		0.97 (0.82-1.14)
Peanuts (g/day)												
0	0.0	9711	696	1.00 (reference)	448	1.00 (reference)	198	1.00 (reference)	241	1.00 (reference)	177	1.00 (reference)
0.1-<5	2.5	10 376	718	0.99 (0.84-1.17)	462	1.00 (0.83-1.20)	216	1.07 (0.85-1.37)	226	0.88 (0.70-1.10)	178	0.97 (0.75-1.24)
5-<10	8.5	3216	197	0.91 (0.71-1.17)	133	0.98 (0.74-1.29)	58	0.99 (0.69-1.43)	70	0.93 (0.66-1.31)	35	0.61 (0.40-0.92)
10+	21.4	5643	382	0.97 (0.79-1.19)	253	1.02 (0.81-1.28)	117	1.13 (0.84-1.51)	131	0.94 (0.71-1.25)	85	0.80 (0.58-1.10)
P-trend				0.704		0.858		0.527		0.944		0.112
Continuous, per 5 g/day increment				1.00 (0.97-1.02)		0.99 (0.96-1.02)		1.00 (0.97-1.04)		0.99 (0.95-1.03)		0.99 (0.95-1.04)
Peanut butter (g/day)												
0	0.0	20 730	1427	1.00 (reference)	929	1.00 (reference)	425	1.00 (reference)	476	1.00 (reference)	347	1.00 (reference)
0.1-<5	1.2	4995	318	0.99 (0.82-1.19)	208	0.98 (0.79-1.20)	94	0.98 (0.75-1.28)	106	0.96 (0.74-1.24)	70	0.91 (0.68-1.22)
5+	9.6	3220	248	1.22 (0.98-1.51)	159	1.21 (0.95-1.54)	70	1.17 (0.86-1.60)	86	1.27 (0.95-1.70)	58	1.14 (0.82-1.59)

(Continued)			Colorectal cancer		Colon cancer		Proximal colon cancer		Distal colon cancer		Rectal cancer	
	Median intake[a]	Person-years	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]
P-trend				0.076		0.125		0.318		0.109		0.429
Continuous, per 5 g/day increment				1.07 (0.99-1.15)		1.06 (0.98-1.15)		1.07 (0.97-1.19)		1.05 (0.95-1.16)		1.01 (0.90-1.13)
Women												
Total nut intake (g/day)												
0	0.0	13 183	629	1.00 (reference)	452	1.00 (reference)	276	1.00 (reference)	163	1.00 (reference)	126	1.00 (reference)
0.1-<5	2.1	12 150	606	1.10 (0.94-1.30)	462	1.18 (0.99-1.41)	261	1.11 (0.90-1.37)	189	1.31 (1.02-1.67)	106	0.95 (0.71-1.27)
5-<10	7.8	3762	149	0.94 (0.73-1.22)	125	1.13 (0.86-1.49)	71	1.09 (0.78-1.51)	49	1.18 (0.81-1.73)	14	0.42 (0.23-0.76)
10+	15.7	4223	190	0.96 (0.75-1.22)	148	1.07 (0.82-1.39)	95	1.13 (0.83-1.54)	51	1.02 (0.70-1.47)	31	0.74 (0.47-1.16)
P-trend				0.458		0.816		0.517		0.782		0.060
Continuous, per 5 g/day increment				1.00 (0.96-1.04)		1.02 (0.97-1.06)		1.02 (0.97-1.07)		1.02 (0.95-1.09)		0.93 (0.83-1.03)
Tree nuts (g/day)												
0	0.0	23 269	1124	1.00 (reference)	835	1.00 (reference)	507	1.00 (reference)	306	1.00 (reference)	207	1.00 (reference)
0.1-<5	1.6	8228	368	0.96 (0.81-1.14)	289	1.02 (0.85-1.23)	159	0.92 (0.74-1.15)	122	1.19 (0.92-1.53)	55	0.80 (0.57-1.11)
5+	8.9	1821	82	0.98 (0.71-1.36)	63	1.03 (0.73-1.46)	37	1.00 (0.66-1.52)	24	1.09 (0.67-1.77)	15	0.99 (0.55-1.79)
P-trend				0.858		0.834		0.895		0.571		0.809
Continuous, per 5 g/day increment				0.98 (0.89-1.07)		0.99 (0.91-1.08)		1.01 (0.91-1.11)		0.97 (0.85-1.10)		0.94 (0.72-1.21)
Peanuts (g/day)												
0	0.0	15 536	733	1.00 (reference)	531	1.00 (reference)	316	1.00 (reference)	201	1.00 (reference)	143	1.00 (reference)
0.1-<5	2.5	12 435	608	1.09 (0.93-1.28)	473	1.18 (0.99-1.40)	278	1.20 (0.97-1.47)	182	1.16 (0.91-1.47)	101	0.93 (0.69-1.25)
5-<10	8.5	2442	96	0.89 (0.66-1.20)	76	1.00 (0.73-1.38)	39	0.88 (0.59-1.31)	33	1.12 (0.72-1.74)	12	0.54 (0.29-1.02)
10+	17.1	2905	137	1.03 (0.79-1.35)	107	1.13 (0.85-1.52)	70	1.27 (0.90-1.78)	36	0.98 (0.65-1.49)	21	0.78 (0.46-1.30)
P-trend				0.849		0.628		0.398		0.908		0.169
Continuous, per 5 g/day increment				1.00 (0.96-1.06)		1.03 (0.97-1.08)		1.03 (0.96-1.09)		1.03 (0.95-1.12)		0.91 (0.80-1.04)
Peanut butter (g/day)												
0	0.0	24 266	1170	1.00 (reference)	866	1.00 (reference)	520	1.00 (reference)	323	1.00 (reference)	214	1.00 (reference)
0.1-<5	1.2	5866	269	1.00 (0.83-1.20)	208	1.05 (0.86-1.29)	119	0.99 (0.77-1.26)	86	1.19 (0.90-1.57)	46	0.91 (0.65-1.30)
5+	6.9	3186	135	0.95 (0.74-1.22)	113	1.08 (0.82-1.41)	64	1.03 (0.74-1.42)	43	1.09 (0.75-1.59)	17	0.63 (0.37-1.09)

(Continued)	Median intake[a]	Person-years	Colorectal cancer		Colon cancer		Proximal colon cancer		Distal colon cancer		Rectal cancer	
			Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]	Cases	Multivariable-adjusted HR (95%CI)[b]
P-trend				0.682		0.559		0.879		0.596		0.092
Continuous, per 5 g/day increment				0.96 (0.85-1.08)		1.01 (0.89-1.14)		0.99 (0.84-1.16)		1.05 (0.89-1.22)		0.78 (0.60-1.02)

[a] Median intake in the subcohort

[b] Adjusted for age (years; continuous), cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5/18.5-<25/25-<30/30+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of colorectal cancer (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

these estimates. In women, no clear associations were observed with peanut butter intake, except for a significant inverse association in mutated *APC* tumors in continuous analyses (HR (95%CI) per 5 g/day increment = 0.44 (0.22-0.90)), which became nonsignificant after FDR correction.

The heterogeneity test was significant for the associations between tree nut intake and the risk of tumors with and without p53 overexpression in women (*P*-heterogeneity = 0.028), although no significant associations were seen for these molecular subtypes.

When combining a truncating *APC* mutation and/or activating *KRAS* mutation and/or p53 overexpression into one endpoint (traditional adenoma-carcinoma pathway), no associations were observed in both sexes, except for a significant positive association for peanut butter intake in men in continuous analyses (HR (95%CI) per 5 g/day increment = 1.18 (1.04-1.33)) (Table 5). However, the FDR-adjusted *P*-value for this continuous estimate was not significant. No relation was observed in colorectal tumors that did not develop through the traditional adenoma-carcinoma pathway. In colorectal tumors with *BRAF* mutation and/or MSI (serrated neoplasia pathway), also no relation with nut intake was observed. In tumors that did not develop through the serrated neoplasia pathway, a significant positive association with peanut butter intake in men in continuous analyses was found (HR (95%CI) per 5 g/day increment = 1.22 (1.07-1.38)), which remained significant after FDR correction. About 74% of the tumors arising from the traditional adenoma-carcinoma pathway were also defined as tumors that did not develop through the serrated neoplasia pathway.

In both sexes, no heterogeneity was observed in associations between CRC tumors that did or did not develop through the traditional adenoma-carcinoma pathway, or between tumors that did or did not develop through the serrated neoplasia pathway.

Discussion

After 20.3 years of follow-up, we observed significant inverse associations between total nut, peanut, and peanut butter intake and rectal cancer risk in women in restricted cubic spline analyses, and the nonlinearity tests were significant for total nut and peanut intake. In men, the inverse nonlinear relations between total nut and peanut intake and rectal cancer risk were borderline significant. However, in categorical and continuous analyses, no significant associations between nut (subtypes) and peanut butter intake and the risk of CRC or its anatomical subtypes were found in men and women. In the analyses of molecular CRC subtypes after 7.3 years of follow-up, peanut butter intake was associated with an increased risk of CRC that did not develop through the serrated neoplasia pathway in men in continuous analyses, but this was not significantly different from the associations with tumors characterized by the serrated neoplasia pathway. Nut and peanut butter intake were not significantly associated with other molecular CRC subtypes in both sexes.

Our results for the anatomical CRC subtypes are partly in line with the results of eight

previous cohort studies (9-16). In five cohort studies, also no significant associations between nut intake and colorectal or colon cancer risk were found in men and/or women (9,10,12,14,15). In another cohort study, an inverse association between peanut product intake and CRC risk was observed in women, but not in men (13). Two other cohorts found inverse associations between nut intake and (distal) colon cancer risk in women (11,16), but not in men (11) or for other anatomical CRC subtypes (11,16). No prospective studies have

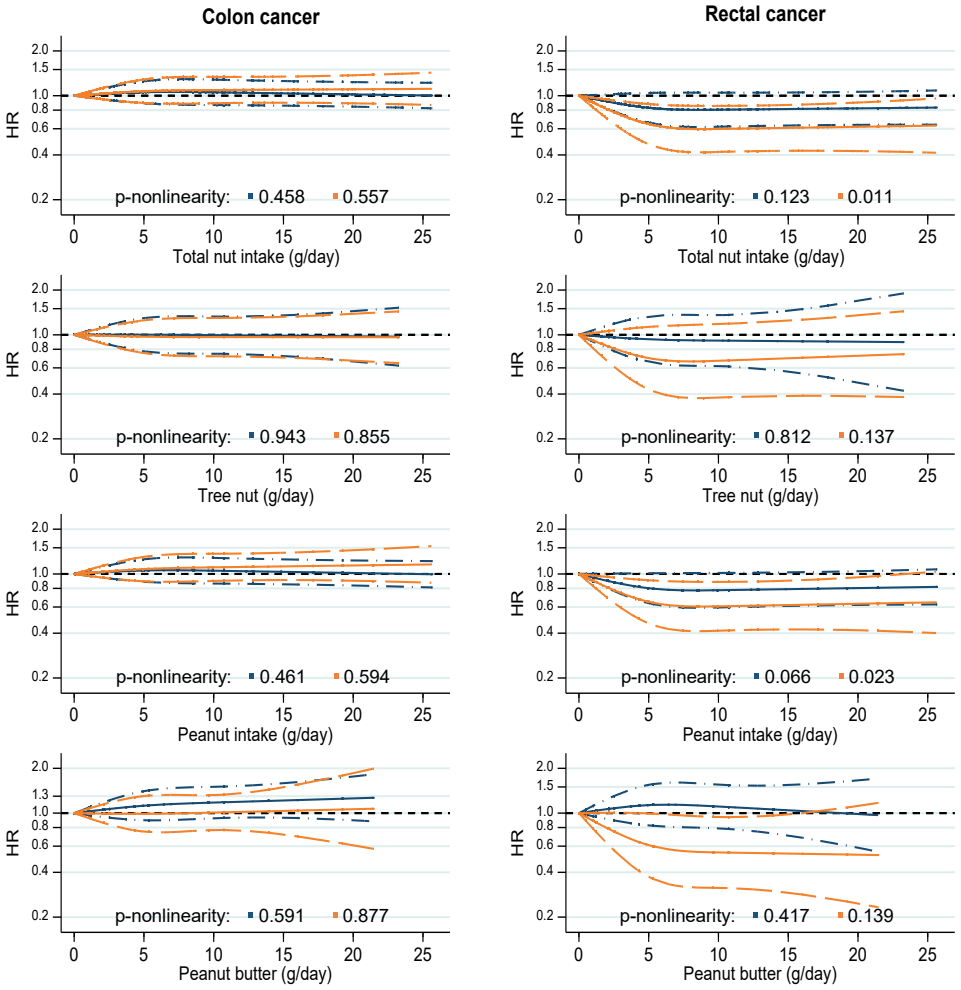

Fig. 1. Restricted cubic spline curves, with three fixed knots at 0, 5, and 10 g nut or peanut butter intake/day; NLCS, 1986-2006. Dark grey lines represent males and light grey lines females. Solid lines represent HRs and dashed lines 95%CIs. HRs were multivariable-adjusted for age (years; continuous), cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5/18.5-<25/25-<30/30+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of colorectal cancer (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

Table 3. Multivariable-adjusted HRs and 95%CI for the relation between nut and peanut butter intake and the risk of molecular subtypes of colorectal cancer in men; NLCS, 1986-1993, excluding the first 2.3 years of follow-up

	Total nuts (g/day)						Tree nuts (g/day)				Peanuts (g/day)					Peanut butter (g/day)				
	0.0	0.1-<5/ 0.1+	5-<10/ 5+	10+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment
Person-years in subcohort	2592	2767/ 5897	1115/ 3129	2014			6175	2314			2913	3001/ 5576	2575			6082	1458/ 2407	949		
Total CRC																				
N cases	115	103	39	55			240	72			124	114	74			226	41	45		
HR[a]	1	0.93	0.90	0.65	0.060/	0.97	1	0.84	0.236/	0.91	1	1.02	0.76	0.072/	0.98	1	0.83	1.44	0.057/	1.14
95%CI	ref	0.69-1.27	0.59-1.37	0.45-0.95	0.352	0.92-1.03	ref	0.62-1.12	0.528	0.71-1.17	ref	0.76-1.37	0.54-1.06	0.352	0.92-1.04	ref	0.57-1.19	0.99-2.09	0.352	1.02-1.29
APC wild-type																				
N cases	73	68	61				154	48			77	76	49			142	31	29		
HR[a]	1	1.01	0.79		0.169/	0.99	1	0.88	0.481/	0.92	1	1.15	0.84	0.264/	1.00	1	0.99	1.48	0.090/	1.12
95%CI	ref	0.70-1.46	0.53-1.16		0.487	0.93-1.06	ref	0.62-1.25	0.705	0.71-1.20	ref	0.81-1.63	0.56-1.27	0.528	0.93-1.07	ref	0.65-1.52	0.94-2.33	0.396	0.97-1.30
Truncating *APC* mutation																				
N cases	42	35	33				86	24			47	38	25			84	10	16		
HR[a]	1	0.81	0.67		0.169/	0.93	1	0.76	0.269/	0.89	1	0.83	0.62	0.113/	0.93	1	0.55	1.36	0.290/	1.18
95%CI	ref	0.50-1.31	0.40-1.13		0.487	0.83-1.04	ref	0.47-1.23	0.528	0.54-1.47	ref	0.52-1.32	0.36-1.09	0.440	0.82-1.05	ref	0.28-1.07	0.76-2.42	0.532	0.99-1.40
P-hetero-geneity						0.922				0.681					0.724					0.319
KRAS wild-type																				
N cases	78	72	28	32			158	52			83	80	47			147	31	32		
HR[a]	1	0.94	0.95	0.54	0.043/	0.94	1	0.90	0.549/	0.92	1	1.05	0.70	0.054/	0.94	1	0.95	1.55	0.047/	1.15
95%CI	ref	0.66-1.34	0.58-1.54	0.34-0.87	0.352	0.87-1.02	ref	0.64-1.27	0.724	0.69-1.22	ref	0.75-1.48	0.47-1.06	0.352	0.87-1.02	ref	0.62-1.45	1.00-2.40	0.352	1.01-1.32
Activating *KRAS* mutation																				
N cases	37	31	11	23			82	20			41	34	27			79	10	13		
HR[a]	1	0.92	0.81	0.91	0.630/	1.02	1	0.71	0.179/	0.89	1	0.95	0.87	0.642/	1.03	1	0.59	1.21	0.557/	1.12
95%CI	ref	0.55-1.55	0.39-1.66	0.51-1.62	0.724	0.94-1.11	ref	0.43-1.17	0.487	0.57-1.39	ref	0.58-1.56	0.51-1.51	0.724	0.95-1.12	ref	0.30-1.17	0.65-2.25	0.724	0.90-1.40
P-hetero-geneity						0.466				0.323					0.613					0.386

(Continued)	Total nuts (g/day)						Tree nutes (g/day)				Peanuts (g/day)					Peanut butter (g/day)				
	0.0	0.1-<5/ 0.1+	5-<10/ 5+	10+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment
No p53 overexpression																				
N cases	45	44	19	20			98	30			50	49	29			96	16	16		
HR[a]	1	1.05	1.12	0.60	0.276/	0.94	1	0.89	0.605/	0.89	1	1.11	0.72	0.149/	0.94	1	0.78	1.18	0.570/	1.01
95%CI	ref	0.67-1.63	0.62-2.04	0.33-1.10	0.528	0.85-1.03	ref	0.58-1.38	0.724	0.60-1.32	ref	0.73-1.68	0.43-1.22	0.487	0.84-1.04	ref	0.44-1.36	0.66-2.09	0.724	0.80-1.27
p53 overexpression																				
N cases	68	58	20	34			138	42			72	64	44			129	23	28		
HR[a]	1	0.87	0.78	0.69	0.120/	1.00	1	0.83	0.323/	0.93	1	0.97	0.79	0.239/	1.00	1	0.80	1.59	0.045/	1.21
95%CI	ref	0.59-1.28	0.46-1.35	0.44-1.10	0.440	0.93-1.07	ref	0.57-1.20	0.547	0.70-1.25	ref	0.67-1.42	0.51-1.20	0.528	0.93-1.08	ref	0.50-1.29	1.01-2.52	0.352	1.06-1.38
P-hetero-geneity						0.669				0.985					0.871					0.746
BRAF wild-type																				
N cases	94	82	75				195	56			102	90	59			182	69			
HR[a]	1	0.90	0.71		0.056/	0.97	1	0.80	0.188/	0.93	1	0.97	0.72	0.070/	0.98	1	1.07		0.668/	1.20
95%CI	ref	0.65-1.26	0.50-1.01		0.352	0.91-1.04	ref	0.57-1.12	0.487	0.71-1.21	ref	0.71-1.33	0.50-1.05	0.352	0.91-1.05	ref	0.79-1.46		0.724	1.06-1.35
BRAF mutation																				
N cases	13	17	14				30	14			14	20	10			34	10			
HR[a]	1	1.42	0.98		0.677/	0.93	1	1.24	0.501/	0.97	1	1.62	0.89	0.446/	0.92	1	0.76		0.468/	0.81
95%CI	ref	0.65-3.09	0.43-2.26		0.724	0.83-1.04	ref	0.67-2.30	0.711	0.63-1.51	ref	0.76-3.47	0.37-2.13	0.701	0.81-1.05	ref	0.37-1.58		0.705	0.48-1.38
P-hetero-geneity						0.573				0.156					0.455					0.513
MSS																				
N cases	88	155					187	56			95	148				182	61			
HR[a]	1	0.86			0.312/	0.97	1	0.82	0.242/	0.86	1	0.93		0.611/	0.98	1	0.95		0.735/	1.17
95%CI	ref	0.64-1.16			0.547	0.91-1.04	ref	0.59-1.14	0.528	0.64-1.17	ref	0.69-1.25		0.724	0.92-1.05	ref	0.69-1.30		0.752	1.03-1.34
MSI																				
N cases	12	18					22	8			13	17				20	10			
HR[a]	1	0.79			0.594/	0.96	1	1.07	0.879/	1.23	1	0.84		0.691/	0.90	1	1.37		0.405/	1.02
95%CI	ref	0.34-1.87			0.724	0.77-1.19	ref	0.45-2.51	0.879	0.93-1.63	ref	0.36-1.96		0.723	0.67-1.21	ref	0.65-2.89		0.660	0.74-1.40
P-hetero-geneity						0.691				0.689					0.671					0.377

[a] Adjusted for age (years; continuous), cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years;

continuous, centered)), BMI (kg/m^2; continuous), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of colorectal cancer (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (g/day; continuous)

[b] FDR-adjusted P-trends are calculated with adjustment for 44 tests

been performed on peanut butter intake. The results of eight case-control studies were also inconclusive (17-24). Differences in study results might be explained by the low case numbers in some studies because of short follow-up periods (9,10,12,13). Furthermore, few studies investigated rectal cancer (9,11,16-18,20,23) or nut subtypes (16,17) separately, and some combined intake of nuts with other foods, like legumes or pulses (9,18,19,21,22).

In the analyses of the anatomical CRC subtypes, we observed no significant associations in continuous analyses, which is probably caused by the nonlinear character of the relations. In restricted cubic spline analyses, which do not assume linearity, we observed significant inverse associations between total nut, peanut, and peanut butter intake and rectal cancer risk in women, and borderline significant inverse associations between total nut and peanut intake and rectal cancer risk in men. The nonlinear association between tree nut intake and rectal cancer risk in women was not significant, probably because of the low tree nut intake.

The significant inverse associations with nut and peanut butter intake in women were seen for rectal cancer, but not for colon cancer, and the test for heterogeneity by anatomical subtype was significant. The colon and rectum have different physiologic and biochemical characteristics, and several studies have observed that associations with lifestyle factors differ between these anatomical regions, indicating that these have distinct etiologies (48,49).

The observed sex differences were also seen in previous analyses on nut consumption and cancer risk in the NLCS (50). One possible explanation might be differences in nut intake. Total nut and peanut butter intake were on average higher in men than in women in our study. Furthermore, the observed sex differences might have a hormonal basis. Phytoestrogens in nuts, which are structurally similar to estrogens, have a relatively high affinity for estrogen receptor-beta (51). Binding of phytoestrogens activates estrogen receptor-beta, thereby promoting apoptosis of colorectal cells (51). However, in previous analyses in the NLCS, we found no significant associations between nut intake and ovarian, endometrial, or estrogen receptor-positive breast cancer (52,53). Lastly, the bowel transit time and frequency of constipation have been reported to be higher in women than in men (54). Consequently, fiber in nuts may have a stronger effect in women by increasing fecal volume, diluting fecal carcinogens, and decreasing the intestinal transit time (7,8).

In the molecular analyses, we observed a positive association in continuous analyses in men between peanut butter intake and colorectal tumors that did not develop through the serrated neoplasia pathway. For tumors that developed through the traditional adenoma-carcinoma pathway, also a positive association with peanut butter intake was observed in continuous analyses in men, although not significant after FDR correction. This can be explained by the fact that 74% of the cases with tumors arising from the traditional adenoma-carcinoma pathway were also defined as tumors that did not develop through the serrated neoplasia pathway. Most molecular and anatomical CRC subtypes were also non-significantly positively associated with peanut butter intake, except for tumors with a *BRAF* mutation, MSI, or without p53 overexpression. The mechanism underlying these

Table 4. Multivariable-adjusted HRs and 95%CI for the relation between nut and peanut butter intake and the risk of molecular subtypes of colorectal cancer in women; NLCS, 1986-1993, excluding the first 2.3 years of follow-up

	Total nuts (g/day)					Tree nuts (g/day)				Peanuts (g/day)					Peanut butter (g/day)				
	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment
Person-years in subcohort	3641	3295/ 5443	2149			6378	2706			4287	3360/ 4797	1437			6643	1599/ 2441	842		
Total CRC																			
N cases	110	104	48			194	68			125	104	33			194	48	20		
HR[a]	1	1.15	0.87	0.339/	0.97	1	0.89	0.487/	0.95	1	1.19	0.92	0.658/	0.98	1	1.13	0.95	0.882/	0.92
95%CI	ref	0.85-1.56	0.59-1.28	0.875	0.86-1.10	ref	0.65-1.22	0.875	0.73-1.23	ref	0.89-1.61	0.60-1.42	0.922	0.86-1.11	ref	0.80-1.60	0.57-1.59	0.970	0.73-1.16
APC wild-type																			
N cases	77	68	35			135	45			86	94				129	51			
HR[a]	1	1.10	0.94	0.716/	1.00	1	0.88	0.483/	0.94	1	1.15		0.397/	1.02	1	1.28		0.165/	1.07
95%CI	ref	0.77-1.57	0.60-1.49	0.922	0.87-1.16	ref	0.61-1.27	0.875	0.65-1.35	ref	0.83-1.60		0.875	0.89-1.17	ref	0.90-1.83		0.875	0.85-1.34
Truncating *APC* mutation																			
N cases	33	36	13			59	23			39	43				65	17			
HR[a]	1	1.27	0.73	0.238/	0.91	1	0.94	0.827/	0.97	1	1.08		0.766/	0.88	1	0.68		0.187/	0.44
95%CI	ref	0.76-2.12	0.36-1.46	0.875	0.75-1.12	ref	0.54-1.64	0.958	0.68-1.37	ref	0.66-1.75		0.922	0.68-1.14	ref	0.38-1.21		0.875	0.22-0.90
P-hetero-geneity					0.630				0.612					0.967					0.193
KRAS wild-type																			
N cases	73	70	33			131	45			83	70	23			131	45			
HR[a]	1	1.19	0.95	0.672/	0.94	1	0.91	0.633/	0.72	1	1.22	1.02	0.987/	0.97	1	1.07		0.733/	0.93
95%CI	ref	0.84-1.71	0.60-1.52	0.922	0.82-1.07	ref	0.62-1.34	0.922	0.44-1.17	ref	0.86-1.74	0.61-1.71	0.987	0.85-1.11	ref	0.74-1.55		0.922	0.70-1.24
Activating *KRAS* mutation																			
N cases	37	34	15			63	23			42	34	10			63	23			
HR[a]	1	1.07	0.72	0.265/	1.02	1	0.88	0.588/	1.08	1	1.15	0.76	0.409/	0.98	1	1.08		0.775/	0.88
95%CI	ref	0.65-1.77	0.38-1.39	0.875	0.83-1.24	ref	0.54-1.41	0.922	0.94-1.25	ref	0.69-1.89	0.37-1.56	0.875	0.76-1.27	ref	0.65-1.77		0.922	0.60-1.30
P-hetero-geneity					0.956				0.837					0.947					0.847

(Continued)	Total nuts (g/day)					Tree nuts (g/day)				Peanuts (g/day)					Peanut butter (g/day)				
	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment	0.0	0.1-<5/ 0.1+	5+	P-trend/ FDR-adjusted P-trend[b]	Per 5 g/day increment
No p53 overexpression																			
N cases	40	49	27			78	38			48	49	19			78	28	10		
HR[a]	1	1.54	1.33	0.517/	1.06	1	1.26	0.278/	1.09	1	1.52	1.35	0.391/	1.06	1	1.66	1.21	0.499/	1.04
95%CI	ref	0.98-2.42	0.78-2.27	0.875	0.95-1.20	ref	0.83-1.90	0.875	0.96-1.22	ref	0.98-2.37	0.76-2.41	0.875	0.93-1.21	Ref	1.05-2.64	0.58-2.53	0.875	0.79-1.37
p53 overexpression																			
N cases	68	55	21			114	30			75	55	14			115	19	10		
HR[a]	1	0.97	0.62	0.084/	0.82	1	0.67	0.083/	0.54	1	1.04	0.68	0.209/	0.86	1	0.75	0.78	0.444/	0.78
95%CI	ref	0.66-1.42	0.36-1.09	0.875	0.68-1.00	ref	0.43-1.05	0.875	0.25-1.18	ref	0.71-1.51	0.36-1.26	0.875	0.69-1.06	ref	0.45-1.24	0.39-1.56	0.875	0.53-1.14
P-hetero-geneity					0.069				0.028					0.166					0.063
BRAF wild-type																			
N cases	86	77	38			151	50			97	104				150	51			
HR[a]	1	1.09	0.88	0.491/	0.99	1	0.84	0.339/	0.99	1	1.10		0.562/	0.99	1	1.02		0.933/	0.86
95%CI	ref	0.78-1.53	0.57-1.37	0.875	0.87-1.13	ref	0.59-1.20	0.875	0.78-1.25	ref	0.80-1.50		0.916	0.86-1.14	ref	0.72-1.44		0.977	0.66-1.12
BRAF mutation																			
N cases	17	23	10			33	17			21	29				37	13			
HR[a]	1	1.70	1.20	0.931/	0.98	1	1.36	0.350/	0.87	1	1.47		0.222/	1.00	1	1.20		0.609/	1.08
95%CI	ref	0.88-3.28	0.53-2.74	0.977	0.79-1.20	ref	0.71-2.60	0.875	0.42-1.83	ref	0.79-2.71		0.875	0.82-1.20	ref	0.60-2.38		0.922	0.63-1.85
P-hetero-geneity					0.510				0.216					0.436					0.932
MSS																			
N cases	88	116				154	50			100	104				154	50			
HR[a]	1	0.99		0.974/	0.99	1	0.81	0.239/	1.01	1	1.05		0.754/	0.98	1	0.97		0.853/	0.91
95%CI	ref	0.73-1.36		0.987	0.86-1.13	ref	0.57-1.15	0.875	0.84-1.22	ref	0.77-1.43		0.922	0.84-1.14	ref	0.68-1.37		0.962	0.70-1.20
MSI																			
N cases	13	25				23	15			16	22				27	11			
HR[a]	1	1.74		0.136/	0.87	1	1.96	0.064/	0.52	1	1.72		0.134/	0.94	1	1.46		0.326/	0.85
95%CI	ref	0.84-3.61		0.875	0.67-1.14	ref	0.96-4.01	0.875	0.28-0.95	ref	0.85-3.50		0.875	0.74-1.20	ref	0.69-3.11		0.875	0.52-1.38
P-hetero-geneity					0.298				0.069					0.431					0.575

[a] Adjusted for age (years; continuous), cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (kg/m^2; continuous), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of colorectal cancer (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (g/day; continuous)

[b] FDR-adjusted P-trends are calculated with adjustment for 44 tests

Table 5. Multivariable-adjusted[a] HRs and 95%CI for the relation between nut and peanut butter intake and the risk of CRC developed through the traditional adenoma-carcinoma pathway (truncating *APC* mutation and/or activating *KRAS* mutation and/or p53 overexpression) and the serrated neoplasia pathway (*BRAF* mutation and/or MSI); NLCS, 1986-1993, excluding the first 2.3 years of follow-up

		Traditional pathway		No traditional pathway		Serrated pathway		No serrated pathway	
	Person-years	N cases	HR (95%CI)	N cases	HR (95%CI)	N cases	HR (95%CI)	N cases	HR (95%CI)
Men									
Total nuts (g/day)									
0.0	2592	95	1 (reference)	19	1 (reference)	19	1 (reference)	81	1 (reference)
0.1-<5	2767	81	0.87 (0.63-1.22)	21	1.24 (0.66-2.32)	22	1.25 (0.64-2.45)	67	0.87 (0.61-1.25)
5+	3129	77	0.73 (0.51-1.03)	16	0.81 (0.39-1.67)	18	0.88 (0.43-1.80)	65	0.72 (0.49-1.05)
P-trend			0.086		0.387		0.502		0.104
FDR-adjusted P-trend[b]			0.458		0.688		0.730		0.458
Per 5 g/day increment			0.97 (0.91-1.04)		0.98 (0.87-1.11)		0.96 (0.86-1.08)		0.96 (0.89-1.04)
P-hetero-geneity					0.729				0.652
Tree nuts (g/day)									
0.0	6175	195	1 (reference)	42	1 (reference)	40	1 (reference)	167	1 (reference)
0.1+	2314	58	0.82 (0.60-1.13)	14	0.98 (0.51-1.89)	19	1.31 (0.76-2.25)	46	0.76 (0.53-1.09)
P-trend			0.230		0.960		0.336		0.136
FDR-adjusted P-trend[b]			0.613		0.983		0.688		0.458
Per 5 g/day increment			0.93 (0.72-1.20)		0.85 (0.47-1.53)		1.09 (0.82-1.46)		0.89 (0.66-1.21)
P-hetero-geneity					0.745				0.093
Peanuts (g/day)									
0.0	2913	103	1 (reference)	20	1 (reference)	20	1 (reference)	88	1 (reference)
0.1-<5 / 0.1+	3001/5576	90	0.95 (0.69-1.31)	23	1.39 (0.76-2.57)	26	1.49 (0.78-2.86)	73	0.92 (0.65-1.30)
5+	2575	60	0.73 (0.50-1.06)	13	0.87 (0.41-1.85)	13	0.83 (0.39-1.79)	52	0.74 (0.50-1.11)
P-trend			0.082		0.484		0.348		0.143
FDR-adjusted P-trend[b]			0.458		0.730		0.688		0.458
Per 5 g/day increment			0.97 (0.91-1.05)		0.99 (0.88-1.12)		0.95 (0.83-1.08)		0.97 (0.89-1.05)
P-hetero-geneity					0.732				0.383
Peanut butter (g/day)									
0.0	6082	185	1 (reference)	40	1 (reference)	42	1 (reference)	158	1 (reference)
0.1+	2407	68	1.02 (0.75-1.38)	16	1.15 (0.61-2.18)	17	1.07 (0.60-1.90)	55	1.00 (0.71-1.40)
P-trend			0.913		0.661		0.820		0.983
FDR-adjusted P-trend[b]			0.983		0.881		0.983		0.983
Per 5 g/day increment			1.18 (1.04-1.33)		0.85 (0.57-1.26)		0.93 (0.67-1.27)		1.22 (1.07-1.38)*
P-hetero-geneity					0.805				0.654
Women									
Total nuts (g/day)									
0.0	3641	86	1 (reference)	24	1 (reference)	23	1 (reference)	78	1 (reference)
0.1-<5	3295	76	1.05 (0.75-1.47)	28	1.55 (0.87-2.78)	28	1.55 (0.87-2.78)	70	1.09 (0.77-1.56)

(Continued)		Traditional pathway		No traditional pathway		Serrated pathway		No serrated pathway	
	Person-years	N cases	HR (95%CI)	N cases	HR (95%CI)	N cases	HR (95%CI)	N cases	HR (95%CI)
5+	2149	34	0.76 (0.48-1.18)	14	1.31 (0.63-2.73)	12	1.03 (0.49-2.16)	31	0.82 (0.51-1.31)
P-trend			0.173		0.647		0.790		0.326
FDR-adjusted P-trend[b]			0.752		0.846		0.903		0.846
Per 5 g/day increment			0.98 (0.85-1.13)		0.93 (0.77-1.12)		0.95 (0.79-1.15)		0.98 (0.84-1.15)
P-hetero-geneity					0.558				0.600
Tree nuts (g/day)									
0.0	6378	149	1 (reference)	45	1 (reference)	44	1 (reference)	132	1 (reference)
0.1+	2706	47	0.78 (0.54-1.13)	21	1.30 (0.73-2.29)	19	1.13 (0.62-2.05)	47	0.92 (0.63-1.33)
P-trend			0.188		0.374		0.687		0.651
FDR-adjusted P-trend[b]			0.752		0.846		0.846		0.846
Per 5 g/day increment			0.98 (0.76-1.27)		0.79 (0.45-1.40)		0.70 (0.30-1.68)		1.04 (0.87-1.24)
P-hetero-geneity					0.222				0.563
Peanuts (g/day)									
0.0	4287	97	1 (reference)	28	1 (reference)	27	1 (reference)	89	1 (reference)
0.1-<5/0.1+	3360/4797	99	1.02 (0.74-1.40)	38	1.53 (0.89-2.63)	26	1.49 (0.82-2.69)	71	1.14 (0.81-1.61)
5+	1437					10	1.34 (0.63-2.87)	19	0.77 (0.45-1.32)
P-trend			0.914		0.126		0.506		0.310
FDR-adjusted P-trend[b]			0.975		0.752		0.846		0.846
Per 5 g/day increment			0.98 (0.85-1.14)		0.95 (0.78-1.16)		0.99 (0.84-1.16)		0.97 (0.80-1.16)
P-hetero-geneity					0.300				0.494
Peanut butter (g/day)									
0.0	6643	149	1 (reference)	45	1 (reference)	47	1 (reference)	134	1 (reference)
0.1+	2441	47	0.92 (0.64-1.32)	21	1.62 (0.93-2.81)	16	1.16 (0.63-2.14)	45	1.00 (0.69-1.45)
P-trend			0.655		0.087		0.623		0.990
FDR-adjusted P-trend[b]			0.846		0.752		0.846		0.990
Per 5 g/day increment			0.82 (0.61-1.10)		1.17 (0.83-1.64)		1.03 (0.62-1.70)		0.84 (0.63-1.13)
P-hetero-geneity					0.222				0.969

[a] Adjusted for age (years; continuous), cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (kg/m^2; continuous), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of colorectal cancer (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (g/day; continuous)

[b] FDR-adjusted P-trends are calculated with adjustment for 16 tests per sex

* P<0.05 after FDR correction

positive associations is not understood, and requires further studies. However, it is known that peanut butter that was sold in the Netherlands in 1986 contained more sodium, trans fatty acids, and vitamin B6, and less niacin than peanuts (5). Trans fatty acids have been hypothesized to increase cancer risk, although evidence for an association between transfatty acid intake and colorectal cancer risk is limited and inconsistent (55,56).

To our knowledge, no previous studies investigated the relation between nut and peanut butter intake and the risk of molecular CRC subtypes. We performed these analyses to account for molecular heterogeneity, which might dilute the relations between nut and peanut butter intake and overall CRC. However, our results should be interpreted cautiously, because it concerns exploratory analyses in which many tests were performed within small groups.

Our analyses were performed using baseline FFQ data only, although food intake might have changed over time. Nevertheless, dietary habits as measured with the FFQ were stable for minimally five years in a reproducibility study (57), and nut intake appeared to be quite constant in a study with repeated measurements (58). Moreover, the NLCS consisted of an older population at baseline, in which dietary habits were relatively stable. Non-differential misclassification of the exposure may have occurred, which potentially attenuated the estimates. Moreover, we cannot exclude residual confounding by (un)measured confounders.

Multiple testing may have resulted in chance findings, especially in the molecular analyses because of their explorative nature, the smaller case numbers, and more unstable results. Therefore, we applied the FDR-correction in the molecular analyses. Because of the short follow-up period of 7.3 years in the molecular analyses, the number of CRC cases was low, which made it impossible to investigate molecular subtypes of colon and rectal cancer separately or different mutation types. Moreover, we only looked at functional mutations, which likely contributed to CRC development. The potential impact of non-functional mutations is not yet understood. Consequently, our findings need to be interpreted carefully.

The prospective design and the long and complete follow-up of the NLCS make selection and information bias unlikely. The detailed information on potential confounders allowed us to extensively control for most known risk factors, although these were only measured at baseline and may have changed over time. Moreover, we were able to investigate nut subtypes separately.

In conclusion, we observed significant nonlinear inverse relations with rectal cancer risk for nut and peanut butter intake in women. In men, nut intake might be nonlinearly associated with a reduced rectal cancer risk, although these associations were borderline significant. Peanut butter intake was associated with an increased risk of CRC that did not develop through the serrated neoplasia pathway in men. However, the results of the molecular analyses should be interpreted cautiously, because this is the first study investigating the relation between nut intake and molecular CRC subtypes, and because many subgroup analyses were performed in small case groups. Therefore, our results need to be replicated in larger studies.

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Supplementary Table 1. Age-adjusted HRs and 95%CI for colorectal cancer and anatomical subtypes in men, according to nut and peanut butter consumption; NLCS, 1986-2006

			Colorectal cancer		Colon cancer		Proximal colon cancer		Distal colon cancer		Rectal cancer	
	Median intake[a]	Person-years	Cases	Age-adjusted HR (95%CI)	Cases	Age-adjusted HR (95%CI)	Cases	Age-adjusted HR (95%CI)	Cases	Age-adjusted HR (95%CI)	Cases	Age-adjusted HR (95%CI)
Total nut intake (g/day)												
0	0.0	8605	614	1.00 (reference)	397	1.00 (reference)	173	1.00 (reference)	218	1.00 (reference)	155	1.00 (reference)
0.1-<5	2.5	9509	668	0.99 (0.84-1.17)	426	0.98 (0.81-1.18)	196	1.04 (0.81-1.32)	213	0.89 (0.71-1.11)	168	0.98 (0.76-1.26)
5-<10	8.5	3896	252	0.92 (0.74-1.15)	170	0.96 (0.75-1.23)	78	1.02 (0.74-1.41)	82	0.84 (0.62-1.13)	48	0.69 (0.48-0.99)
10+	21.4	6936	459	0.96 (0.80-1.16)	303	0.99 (0.81-1.21)	142	1.08 (0.83-1.41)	155	0.91 (0.71-1.17)	104	0.85 (0.64-1.12)
P-trend				0.652		0.988		0.590		0.652		0.171
Continuous, per 5 g/day increment				1.00 (0.97-1.02)		0.99 (0.97-1.02)		1.00 (0.97-1.04)		0.99 (0.95-1.02)		1.00 (0.96-1.04)
Tree nuts (g/day)												
0	0.0	20837	1453	1.00 (reference)	935	1.00 (reference)	421	1.00 (reference)	488	1.00 (reference)	346	1.00 (reference)
0.1-<5	1.6	6615	439	0.95 (0.81-1.12)	289	0.97 (0.81-1.16)	130	0.97 (0.77-1.23)	147	0.95 (0.76-1.18)	109	0.99 (0.77-1.26)
5+	8.9	1494	101	0.97 (0.71-1.31)	72	1.07 (0.77-1.49)	38	1.25 (0.84-1.87)	33	0.94 (0.61-1.43)	20	0.80 (0.49-1.33)
P-trend				0.729		0.754		0.317		0.699		0.406
Continuous, per 5 g/day increment				0.98 (0.89-1.07)		1.00 (0.91-1.10)		1.03 (0.93-1.15)		0.96 (0.85-1.09)		0.96 (0.82-1.12)
Peanuts (g/day)												
0	0.0	9711	696	1.00 (reference)	448	1.00 (reference)	198	1.00 (reference)	241	1.00 (reference)	177	1.00 (reference)
0.1-<5	2.5	10376	718	0.99 (0.84-1.16)	462	0.99 (0.83-1.19)	216	1.07 (0.85-1.34)	226	0.89 (0.72-1.11)	178	0.95 (0.75-1.21)
5-<10	8.5	3216	197	0.90 (0.71-1.13)	133	0.95 (0.73-1.23)	58	0.96 (0.68-1.35)	70	0.91 (0.67-1.25)	35	0.61 (0.41-0.91)
10+	21.4	5643	382	1.00 (0.82-1.21)	253	1.04 (0.84-1.28)	117	1.11 (0.84-1.46)	131	0.98 (0.76-1.27)	85	0.85 (0.63-1.14)
P-trend				0.933		0.727		0.563		0.883		0.190
Continuous, per 5 g/day increment				1.00 (0.97-1.02)		0.99 (0.97-1.02)		1.00 (0.97-1.04)		0.99 (0.95-1.02)		1.00 (0.96-1.04)
Peanut butter (g/day)												
0	0.0	20730	1427	1.00 (reference)	929	1.00 (reference)	425	1.00 (reference)	476	1.00 (reference)	347	1.00 (reference)
0.1-<5	1.2	4995	318	0.96 (0.81-1.16)	208	0.98 (0.80-1.20)	94	0.98 (0.76-1.28)	106	0.96 (0.75-1.23)	70	0.85 (0.64-1.14)
5+	9.6	3220	248	1.16 (0.94-1.44)	159	1.16 (0.91-1.46)	70	1.13 (0.83-1.53)	86	1.21 (0.91-1.60)	58	1.10 (0.80-1.51)
P-trend				0.150		0.223		0.429		0.188		0.558
Continuous, per 5 g/day increment				1.03 (0.96-1.11)		1.03 (0.95-1.11)		1.04 (0.95-1.15)		1.02 (0.93-1.12)		0.99 (0.88-1.10)

[a] Median intake in the subcohort

Supplementary Table 2. Age-adjusted HRs and 95%CI for colorectal cancer and anatomical subtypes in women, according to nut and peanut butter consumption; NLCS, 1986-2006

			Colorectal cancer		Colon cancer		Proximal colon cancer		Distal colon cancer		Rectal cancer	
	Median intake[a]	Person-years	Cases	Age-adjusted HR (95%CI)	Cases	Age-adjusted HR (95%CI)	Cases	Age-adjusted HR (95%CI)	Cases	Age-adjusted HR (95%CI)	Cases	Age-adjusted HR (95%CI)
Total nut intake (g/day)												
0	0.0	13183	629	1.00 (reference)	452	1.00 (reference)	276	1.00 (reference)	163	1.00 (reference)	126	1.00 (reference)
0.1-<5	2.1	12150	606	1.09 (0.93-1.28)	462	1.16 (0.98-1.38)	261	1.09 (0.89-1.33)	189	1.29 (1.02-1.64)	106	0.94 (0.71-1.25)
5-<10	7.8	3762	149	0.91 (0.72-1.16)	125	1.08 (0.83-1.39)	71	1.03 (0.75-1.40)	49	1.12 (0.78-1.61)	14	0.42 (0.23-0.75)
10+	15.7	4223	190	1.00 (0.80-1.25)	148	1.09 (0.85-1.39)	95	1.16 (0.88-1.55)	51	1.01 (0.71-1.44)	31	0.80 (0.52-1.22)
P-trend				0.628		0.703		0.392		0.762		0.087
Continuous, per 5 g/day increment				1.00 (0.96-1.04)		1.02 (0.97-1.06)		1.02 (0.97-1.07)		1.01 (0.95-1.08)		0.94 (0.84-1.04)
Tree nuts (g/day)												
0	0.0	23269	1124	1.00 (reference)	835	1.00 (reference)	507	1.00 (reference)	306	1.00 (reference)	207	1.00 (reference)
0.1-<5	1.6	8228	368	0.95 (0.81-1.11)	289	1.00 (0.84-1.19)	159	0.91 (0.74-1.13)	122	1.14 (0.90-1.45)	55	0.77 (0.56-1.05)
5+	8.9	1821	82	0.97 (0.71-1.32)	63	1.00 (0.72-1.40)	37	0.98 (0.66-1.45)	24	1.03 (0.64-1.63)	15	0.96 (0.54-1.69)
P-trend				0.743		0.984		0.786		0.740		0.647
Continuous, per 5 g/day increment				0.97 (0.88-1.06)		0.98 (0.90-1.07)		1.00 (0.90-1.10)		0.96 (0.84-1.09)		0.92 (0.70-1.21)
Peanuts (g/day)												
0	0.0	15536	733	1.00 (reference)	531	1.00 (reference)	316	1.00 (reference)	201	1.00 (reference)	143	1.00 (reference)
0.1-<5	2.5	12435	608	1.10 (0.94-1.28)	473	1.18 (1.00-1.39)	278	1.18 (0.97-1.44)	182	1.17 (0.93-1.47)	101	0.92 (0.69-1.22)
5-<10	8.5	2442	96	0.92 (0.69-1.22)	76	1.01 (0.74-1.37)	39	0.90 (0.61-1.31)	33	1.11 (0.73-1.68)	12	0.57 (0.31-1.07)
10+	17.1	2905	137	1.06 (0.83-1.37)	107	1.15 (0.88-1.52)	70	1.29 (0.94-1.78)	36	0.99 (0.67-1.48)	21	0.82 (0.50-1.35)
P-trend				0.910		0.490		0.281		0.955		0.235
Continuous, per 5 g/day increment				1.01 (0.96-1.06)		1.03 (0.98-1.08)		1.03 (0.97-1.09)		1.03 (0.95-1.10)		0.93 (0.82-1.05)
Peanut butter (g/day)												
0	0.0	24266	1170	1.00 (reference)	866	1.00 (reference)	520	1.00 (reference)	323	1.00 (reference)	214	1.00 (reference)
0.1-<5	1.2	5866	269	0.99 (0.82-1.18)	208	1.03 (0.85-1.26)	119	0.99 (0.79-1.26)	86	1.13 (0.86-1.47)	46	0.92 (0.65-1.29)
5+	6.9	3186	135	0.91 (0.71-1.16)	113	1.03 (0.80-1.33)	64	0.98 (0.72-1.33)	43	1.03 (0.72-1.48)	17	0.62 (0.37-1.04)
P-trend				0.431		0.816		0.890		0.809		0.070
Continuous, per 5 g/day increment				0.95 (0.85-1.06)		1.00 (0.89-1.12)		0.98 (0.84-1.15)		1.03 (0.88-1.20)		0.78 (0.60-1.02)

[a] Median intake in the subcohort

Supplementary Table 3. Multivariable-adjusted HRs and 95%CI for colon cancer according to total nut intake in strata of potential effect modifiers in men and women; NLCS, 1986-2006

	Men					Women				
	Total nut consumption (g/day)			P-trend	P-interaction	Total nut consumption (g/day)			P-trend	P-interaction
	0 g/day	0.1-<5 g/day	5+ g/day			0 g/day	0.1-<5 g/day	5+ g/day		
Overall										
Cases/person-years	394/8572	425/9484	473/10813			445/12925	457/12047	270/7900		
HR (95%CI)[a]	1 (ref)	0.99 (0.81-1.20)	0.97 (0.80-1.18)	0.777		1 (ref)	1.18 (0.99-1.41)	1.10 (0.89-1.37)	0.645	
Body mass index										
18.5-<25 kg/m^2										
Cases/person-years	181/4356	214/5391	237/5835			218/6697	248/6426	171/5163		
HR (95%CI)[a]	1 (ref)	0.93 (0.70-1.23)	0.98 (0.75-1.30)	0.899	0.836	1 (ref)	1.29 (1.00-1.65)	1.15 (0.86-1.53)	0.653	0.626
25+ kg/m^2										
Cases/person-years	213/4216	211/4093	236/4978			227/6229	209/5621	99/2737		
HR (95%CI)[a]	1 (ref)	1.04 (0.79-1.38)	0.93 (0.70-1.24)	0.504		1 (ref)	1.09 (0.83-1.42)	1.06 (0.75-1.49)	0.822	
Nonoccupational physical activity										
≤30 min/day										
Cases/person-years	74/1272	54/1541	65/1790			124/3415	124/2470	72/1354		
HR (95%CI)[a]	1 (ref)	0.61 (0.36-1.04)	0.68 (0.40-1.14)	0.372	0.074	1 (ref)	1.39 (0.97-1.99)	1.43 (0.93-2.21)	0.165	0.487
>30-≤60 min/day										
Cases/person-years	113/3097	132/2798	167/3404			132/4008	134/3786	91/2730		
HR (95%CI)[a]	1 (ref)	1.35 (0.96-1.92)	1.49 (1.06-2.09)	0.050		1 (ref)	1.22 (0.88-1.71)	1.20 (0.80-1.80)	0.487	
>60-≤90 min/day										
Cases/person-years	77/1480	95/1924	100/2345			108/2861	106/3011	64/1904		
HR (95%CI)[a]	1 (ref)	1.05 (0.65-1.69)	0.77 (0.48-1.24)	0.173		1 (ref)	0.97 (0.66-1.41)	0.93 (0.59-1.47)	0.782	
>90 min/day										
Cases/person-years	130/2723	144/3221	141/3274			81/2642	93/2780	43/1911		
HR (95%CI)[a]	1 (ref)	0.83 (0.57-1.19)	0.77 (0.52-1.12)	0.262		1 (ref)	1.05 (0.70-1.60)	0.75 (0.44-1.28)	0.198	
Cigarette smoking status										
Never										
Cases/person-years	59/1251	54/1570	57/1440			286/8206	267/7393	145/4174		
HR (95%CI)[a]	1 (ref)	0.91 (0.50-1.65)	0.95 (0.49-1.83)	0.962	0.671	1 (ref)	1.07 (0.85-1.34)	1.09 (0.82-1.45)	0.601	0.015
Former										

(Continued)	Men					Women				
	Total nut consumption (g/day)			P-trend	P-interaction	Total nut consumption (g/day)			P-trend	P-interaction
Cases/person-years	226/4339	256/5132	307/6097			81/1925	118/2668	56/2194		
HR (95%CI)[a]	1 (ref)	1.00 (0.76-1.30)	1.00 (0.78-1.30)	0.955		1 (ref)	1.40 (0.89-2.22)	0.79 (0.48-1.32)	0.075	
Current										
Cases/person-years	109/2981	115/2782	109/3276			78/2794	72/1986	69/1532		
HR (95%CI)[a]	1 (ref)	1.08 (0.75-1.54)	0.91 (0.62-1.34)	0.490		1 (ref)	1.28 (0.82-1.99)	1.73 (1.05-2.86)	0.041	
Alcohol consumption										
0 g/day										
Cases/person-years	76/2121	47/1188	36/751			179/5416	133/3355	50/1441		
HR (95%CI)[a]	1 (ref)	1.04 (0.61-1.75)	1.37 (0.76-2.47)	0.287	0.643	1 (ref)	1.22 (0.89-1.66)	1.04 (0.67-1.62)	0.883	0.870
0.1-<15 g/day										
Cases/person-years	180/3683	223/5229	216/4906			216/6051	267/7212	169/5121		
HR (95%CI)[a]	1 (ref)	0.89 (0.67-1.18)	0.94 (0.70-1.25)	0.884		1 (ref)	1.14 (0.89-1.45)	1.03 (0.77-1.37)	0.883	
≥15 g/day										
Cases/person-years	138/2768	155/3067	221/5156			50/1458	57/1479	51/1338		
HR (95%CI)[a]	1 (ref)	0.98 (0.70-1.37)	0.88 (0.64-1.19)	0.348		1 (ref)	1.50 (0.81-2.78)	1.74 (0.89-3.40)	0.164	
Educational level										
Low										
Cases/person-years	180/4237	152/4237	168/3670			264/8063	242/6252	121/3601		
HR (95%CI)[a]	1 (ref)	0.88 (0.66-1.18)	1.12 (0.82-1.52)	0.278	0.157	1 (ref)	1.23 (0.97-1.56)	1.08 (0.79-1.47)	0.819	0.317
Medium										
Cases/person-years	147/2840	160/3378	172/4339			146/4099	178/4479	113/3256		
HR (95%CI)[a]	1 (ref)	0.93 (0.66-1.30)	0.80 (0.57-1.11)	0.161		1 (ref)	1.29 (0.94-1.76)	1.13 (0.78-1.64)	0.832	
High										
Cases/person-years	67/1495	113/1869	133/2805			35/764	37/1316	36/1043		
HR (95%CI)[a]	1 (ref)	1.21 (0.75-1.95)	1.11 (0.71-1.75)	0.922		1 (ref)	0.69 (0.35-1.39)	0.90 (0.43-1.90)	0.867	
Family history of colorectal cancer										
No										
Cases/person-years	358/8052	380/9015	431/10160			393/12202	408/11287	247/7431		
HR (95%CI)[a]	1 (ref)	0.94 (0.77-1.16)	0.97 (0.79-1.18)	0.894	0.367	1 (ref)	1.20 (0.99-1.44)	1.15 (0.92-1.44)	0.398	0.368
Yes										
Cases/person-years	36/520	45/468	42/653			52/723	49/760	23/469		
HR (95%CI)[a]	1 (ref)	0.99 (0.38-2.57)	0.63 (0.23-1.76)	0.323		1 (ref)	0.92 (0.40-2.08)	0.87 (0.32-2.36)	0.804	

[a] Adjusted for age (years; continuous), cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (18.5-<25/25+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of colorectal cancer (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

Supplementary Table 4. Multivariable-adjusted HRs and 95%CI for rectal cancer according to total nut intake in strata of potential effect modifiers in men and women; NLCS, 1986-2006

	Men					Women				
	Total nut consumption (g/day)			P-trend	P-interaction	Total nut consumption (g/day)			P-trend	P-interaction
	0 g/day	0.1-<5 g/day	5+ g/day			0 g/day	0.1-<5 g/day	5+ g/day		
Overall										
Cases/person-years	155/8572	167/9484	152/10813			126/12925	105/12047	44/7900		
HR (95%CI)[a]	1 (ref)	1.01 (0.78-1.31)	0.78 (0.60-1.03)	0.043		1 (ref)	0.94 (0.70-1.27)	0.58 (0.39-0.87)	0.005	
Body mass index										
18.5-<25 kg/m^2										
Cases/person-years	86/4356	68/5391	82/5835			66/6697	61/6426	27/5163		
HR (95%CI)[a]	1 (ref)	0.63 (0.44-0.92)	0.70 (0.48-1.01)	0.250	0.002	1 (ref)	1.07 (0.72-1.60)	0.57 (0.34-0.96)	0.006	0.639
25+ kg/m^2										
Cases/person-years	69/4216	99/4093	70/4978			60/6229	44/5621	17/2737		
HR (95%CI)[a]	1 (ref)	1.52 (1.04-2.21)	0.85 (0.56-1.30)	0.079		1 (ref)	0.83 (0.53-1.30)	0.63 (0.34-1.15)	0.145	
Nonoccupational physical activity										
≤30 min/day										
Cases/person-years	23/1272	20/1541	24/1790			40/3415	29/2470	6/1354		
HR (95%CI)[a]	1 (ref)	0.82 (0.38-1.76)	0.94 (0.44-1.98)	0.971	0.702	1 (ref)	1.06 (0.60-1.88)	0.33 (0.13-0.85)	0.015	0.431
>30-≤60 min/day										
Cases/person-years	49/3097	47/2798	50/3404			44/4008	29/3786	12/2730		
HR (95%CI)[a]	1 (ref)	1.15 (0.71-1.85)	1.12 (0.69-1.80)	0.751		1 (ref)	0.77 (0.45-1.32)	0.45 (0.21-0.94)	0.040	
>60-≤90 min/day										
Cases/person-years	26/1480	41/1924	32/2345			22/2861	27/3011	17/1904		
HR (95%CI)[a]	1 (ref)	1.58 (0.84-2.98)	0.77 (0.39-1.50)	0.122		1 (ref)	1.17 (0.59-2.32)	1.27 (0.58-2.72)	0.603	
>90 min/day										
Cases/person-years	57/2723	59/3221	46/3274			20/2642	20/2780	9/1911		
HR (95%CI)[a]	1 (ref)	0.74 (0.46-1.19)	0.54 (0.33-0.89)	0.025		1 (ref)	0.83 (0.39-1.76)	0.49 (0.19-1.27)	0.143	
Cigarette smoking status										
Never										
Cases/person-years	22/1251	20/1570	14/1440			65/8206	61/7393	24/4174		
HR (95%CI)[a]	1 (ref)	0.74 (0.32-1.70)	0.54 (0.21-1.41)	0.242	0.582	1 (ref)	1.08 (0.74-1.57)	0.76 (0.45-1.28)	0.247	0.521
Former										

(Continued)	Men					Women				
	Total nut consumption (g/day)			P-trend	P-interaction	Total nut consumption (g/day)			P-trend	P-interaction
Cases/person-years	76/4339	95/5132	99/6097			27/1925	21/2668	12/2194		
HR (95%CI)[a]	1 (ref)	1.05 (0.73-1.51)	0.88 (0.61-1.26)	0.339		1 (ref)	0.60 (0.26-1.37)	0.37 (0.15-0.94)	0.053	
Current										
Cases/person-years	57/2981	52/2782	39/3276			34/2794	23/1986	8/1532		
HR (95%CI)[a]	1 (ref)	1.03 (0.65-1.63)	0.68 (0.40-1.15)	0.096		1 (ref)	1.02 (0.55-1.91)	0.43 (0.18-1.04)	0.050	
Alcohol consumption										
0 g/day										
Cases/person-years	28/2121	14/1188	13/751			51/5416	26/3355	9/1441		
HR (95%CI)[a]	1 (ref)	1.02 (0.45-2.29)	1.67 (0.72-3.89)	0.214	0.441	1 (ref)	0.83 (0.50-1.40)	0.60 (0.27-1.29)	0.191	0.872
0.1-<15 g/day										
Cases/person-years	66/3683	85/5229	62/4906			59/6051	65/7212	25/5121		
HR (95%CI)[a]	1 (ref)	0.94 (0.64-1.38)	0.74 (0.50-1.11)	0.125		1 (ref)	0.92 (0.61-1.39)	0.49 (0.29-0.84)	0.006	
≥15 g/day										
Cases/person-years	61/2768	68/3067	77/5156			16/1458	14/1479	10/1338		
HR (95%CI)[a]	1 (ref)	1.05 (0.69-1.60)	0.71 (0.47-1.07)	0.043		1 (ref)	1.06 (0.40-2.80)	1.28 (0.47-3.53)	0.608	
Educational level										
Low										
Cases/person-years	94/4237	82/4237	65/3670			71/8063	54/6252	24/3601		
HR (95%CI)[a]	1 (ref)	0.89 (0.61-1.28)	0.75 (0.50-1.12)	0.175	0.404	1 (ref)	1.02 (0.69-1.51)	0.74 (0.44-1.25)	0.229	0.583
Medium										
Cases/person-years	47/2840	52/3378	57/4339			49/4099	40/4479	17/3256		
HR (95%CI)[a]	1 (ref)	0.95 (0.59-1.54)	0.83 (0.51-1.34)	0.421		1 (ref)	0.82 (0.49-1.38)	0.49 (0.25-0.96)	0.039	
High										
Cases/person-years	14/1495	33/1869	30/2805			6/764	11/1316	3/1043		
HR (95%CI)[a]	1 (ref)	1.70 (0.81-3.59)	1.04 (0.48-2.28)	0.427		1 (ref)	0.99 (0.23-4.17)	0.27 (0.04-1.71)	0.102	
Family history of colorectal cancer										
No										
Cases/person-years	146/8052	153/9015	143/10160			113/12202	98/11287	37/7431		
HR (95%CI)[a]	1 (ref)	0.97 (0.74-1.27)	0.78 (0.59-1.03)	0.059	0.354	1 (ref)	1.01 (0.74-1.37)	0.57 (0.37-0.87)	0.005	0.270
Yes										
Cases/person-years	9/520	14/468	9/653			13/723	7/760	7/469		
HR (95%CI)[a]	1 (ref)	1.47 (0.23-9.54)	0.43 (0.05-3.66)	0.270		1 (ref)	0.20 (0.03-1.23)	0.71 (0.17-3.06)	0.913	

[a] Adjusted for age (years; continuous), cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (18.5-<25/25+ kg/m^2), nonoccupational physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), educational level (low/medium/high), family history of colorectal cancer (no/yes), total energy intake (kcal/day; continuous), and alcohol consumption (0/0.1-<5/5-<15/15-<30/30+ g/day)

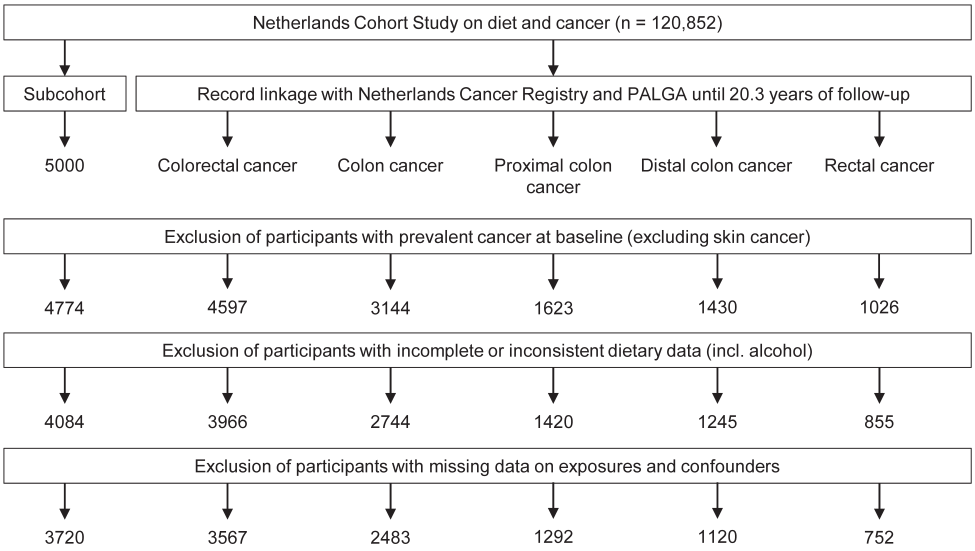
Supplementary Table 5. Median total nut intake of colon and rectal cancer cases diagnosed over the follow-up period; NLCS, 1986-2006

	Colon cancer				Rectal cancer			
	Men		Women		Men		Women	
Time between inclusion (September 1986) and colorectal cancer diagnosis (years)	n	Median total nut intake (g/day) (IQR)	n	Median total nut intake (g/day) (IQR)	n	Median total nut intake (g/day) (IQR)	n	Median total nut intake (g/day) (IQR)
0-<2	72	3.6 (0.0-10.7)	61	2.0 (0.0-5.8)	33	2.5 (0.0-5.8)	23	0.0 (0.0-1.0)
2-<4	71	4.3 (0.0-9.0)	82	1.0 (0.0-4.3)	46	2.3 (0.0-8.5)	23	0.9 (0.0-2.5)
4-<6	105	2.5 (0.0-7.8)	87	1.0 (0.0-4.3)	43	2.0 (0.0-7.0)	23	2.0 (0.0-4.9)
6-<8	129	2.0 (0.0-7.4)	120	1.0 (0.0-4.5)	45	0.0 (0.0-3.6)	26	1.0 (0.0-4.5)
8-<10	137	3.0 (0.5-8.5)	118	1.0 (0.0-4.3)	46	4.3 (1.0-13.2)	20	1.2 (0.0-3.2)
10-<12	167	2.5 (0.0-10.7)	102	1.4 (0.0-4.9)	56	2.3 (0.0-17.2)	29	0.8 (0.0-2.5)
12-<14	145	2.6 (0.0-8.5)	130	1.8 (0.0-4.3)	62	2.0 (0.0-4.9)	30	0.4 (0.0-2.5)
14-<16	146	3.9 (0.0-10.7)	147	1.8 (0.0-5.1)	48	3.3 (1.0-9.5)	37	1.3 (0.0-4.3)
16-<18	163	4.5 (0.0-12.8)	144	2.0 (0.0-6.2)	52	4.2 (0.0-10.7)	25	2.1 (0.0-3.6)
18+	161	3.3 (0.0-9.0)	196	1.8 (0.0-4.9)	44	3.6 (0.0-11.7)	41	1.0 (0.0-2.5)
p[a]		0.072		0.459		0.013		0.380

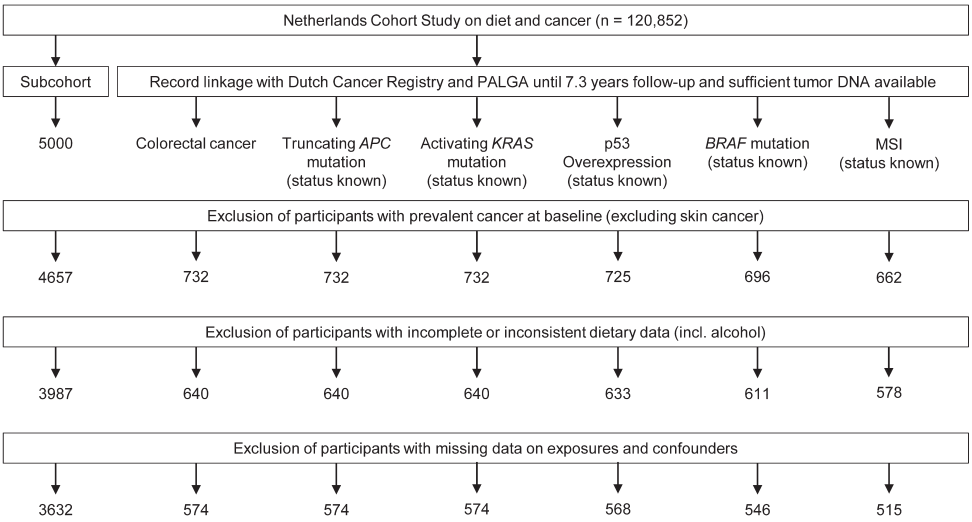
[a] P-values based on Kruskal-Wallis tests

Supplementary Table 6. Baseline characteristics of subcohort members and cases per molecular subtype of colorectal cancer; NLCS, 1986-1993, excluding the first 2.3 years of follow-up

	Subcohort	*APC* wild-type	Truncating *APC* mutation	*KRAS* wild-type	Activating *KRAS* mutation	No p53 over-expression	p53 over-expression	*BRAF* wild-type	*BRAF* mutation	MSS	MSI
Men											
N	1773	202	110	210	102	128	180	251	44	243	30
Age (years)	61.1 (4.2)	63.0 (4.3)	62.5 (3.9)	62.6 (4.2)	63.2 (4.2)	63.2 (4.1)	62.6 (4.2)	62.9 (4.2)	62.1 (4.3)	62.8 (4.1)	63.6 (4.5)
Body Mass Index (kg/m^2)	24.9 (2.6)	25.3 (2.7)	25.4 (3.0)	25.2 (2.8)	25.6 (2.9)	25.5 (3.1)	25.2 (2.6)	25.4 (2.9)	25.1 (2.4)	25.4 (2.9)	24.9 (2.2)
Ever cigarette smoker (%)	86.2	89.1	88.2	89.0	88.2	88.3	88.9	88.8	84.1	87.7	90.0
University or higher vocational education (%)	20.3	20.3	23.6	24.3	15.7	20.3	21.7	20.3	34.1	22.6	26.7
Non-occupational physical activity (min/day)	80.9 (67.2)	86.6 (70.5)	84.2 (82.4)	94.3 (84.5)	68.1 (44.6)	89.7 (88.7)	81.9 (59.2)	82.2 (70.7)	99.1 (89.4)	81.5 (72.2)	106.1 (96.8)
Family history of colorectal cancer (%)	5.6	12.9	12.7	12.9	12.8	14.8	11.7	13.9	9.1	14.4	10.0
Daily energy intake (kcal)	2169 (498)	2131 (418)	2134 (499)	2131 (464)	2136 (415)	2163 (458)	2107 (438)	2128 (460)	2151 (352)	2135 (455)	2151 (412)
Total nut intake (g/day)	8.0 (13.7)	7.5 (14.8)	6.1 (11.3)	6.3 (11.7)	8.5 (17.0)	6.2 (10.9)	7.6 (15.5)	7.1 (14.3)	6.1 (8.6)	7.1 (13.7)	6.5 (14.0)
Tree nut intake (g/day)	1.0 (3.4)	0.9 (3.1)	0.8 (3.6)	0.9 (3.3)	0.8 (3.2)	0.8 (3.0)	0.9 (3.5)	0.9 (3.5)	1.0 (2.8)	0.8 (2.9)	1.9 (6.6)
Peanut intake (g/day)	7.0 (12.9)	6.6 (14.1)	5.3 (10.6)	5.4 (10.7)	7.7 (16.7)	5.4 (10.2)	6.7 (14.7)	6.2 (13.5)	5.2 (8.0)	6.3 (13.1)	4.6 (11.0)
Peanut butter intake (g/day)	1.4 (4.2)	1.7 (4.3)	1.9 (5.0)	1.8 (4.5)	1.6 (4.7)	1.3 (3.6)	2.1 (5.2)	2.0 (5.0)	0.9 (2.4)	1.8 (4.9)	1.3 (2.7)
Alcohol intake (g/day)	15.0 (16.9)	15.3 (15.6)	17.1 (18.4)	16.0 (17.2)	15.9 (15.4)	14.8 (13.2)	16.7 (18.6)	16.5 (17.2)	14.0 (14.7)	16.9 (17.3)	11.9 (13.4)
Women											
N	1859	180	82	176	86	116	144	201	50	204	38
Age (years)	61.4 (4.2)	62.9 (3.9)	62.5 (4.0)	62.6 (4.0)	63.1 (3.8)	63.1 (3.9)	62.6 (4.0)	62.8 (3.9)	62.8 (4.0)	62.7 (3.9)	63.6 (4.3)
Body Mass Index (kg/m^2)	25.0 (3.5)	25.1 (3.4)	26.0 (3.7)	25.4 (3.4)	25.5 (3.7)	25.4 (3.5)	25.4 (3.5)	25.5 (3.6)	25.4 (3.5)	25.4 (3.5)	25.9 (4.2)
Ever cigarette smoker (%)	40.9	39.5	36.6	40.3	34.9	43.1	34.0	37.8	46.0	36.8	44.7
University or higher vocational education (%)	9.5	6.7	14.6	8.0	11.6	7.8	9.7	10.5	4.0	10.3	5.3
Non-occupational physical activity (min/day)	65.6 (50.8)	61.9 (46.3)	57.6 (41.8)	61.5 (43.5)	58.8 (47.9)	59.2 (40.9)	61.5 (48.2)	59.0 (43.4)	68.5 (52.2)	59.3 (43.7)	64.5 (55.7)
Family history of colorectal cancer (%)	6.1	10.0	9.8	11.4	7.0	12.9	7.6	10.0	10.0	11.3	7.9
Daily energy intake (kcal)	1685 (390)	1633 (358)	1728 (419)	1639 (374)	1710 (390)	1696 (405)	1639 (359)	1671 (397)	1620 (309)	1663 (387)	1638 (362)
Total nut intake (g/day)	4.4 (8.5)	3.9 (10.5)	3.4 (7.1)	3.3 (6.4)	4.5 (13.9)	5.2 (13.1)	2.6 (4.9)	3.9 (10.3)	3.7 (6.9)	3.8 (10.3)	2.8 (4.7)
Tree nut intake (g/day)	1.1 (4.0)	0.8 (3.3)	1.0 (3.0)	0.6 (1.9)	1.5 (4.9)	1.4 (4.4)	0.5 (1.7)	1.0 (3.5)	0.8 (2.6)	1.0 (3.6)	0.4 (0.7)
Peanut intake (g/day)	3.3 (7.0)	3.0 (8.0)	2.4 (5.5)	2.7 (5.6)	3.1 (9.9)	3.8 (9.7)	2.1 (4.5)	2.9 (7.9)	2.9 (5.2)	2.8 (7.7)	2.4 (4.7)
Peanut butter intake (g/day)	1.2 (3.6)	1.1 (3.3)	0.4 (1.2)	0.9 (2.9)	0.9 (2.7)	1.2 (3.5)	0.7 (2.2)	0.8 (2.4)	1.0 (4.1)	0.9 (3.0)	0.7 (1.5)
Alcohol intake (g/day)	5.9 (9.3)	6.0 (11.3)	5.4 (10.3)	5.9 (11.5)	5.7 (9.9)	6.3 (10.5)	5.4 (11.3)	5.5 (11.0)	7.8 (11.9)	5.2 (9.3)	8.9 (18.1)



Supplementary Figure 1. Flow diagram of the number of colorectal cancer cases and subcohort members; NLCS, 1986-2006



Supplementary Figure 2. Flow diagram of the number of colorectal cancer cases per molecular subtype and subcohort members; NLCS, 1986-1993, excluding the first 2.3 years of follow-up

Chapter 4

Total nut, tree nut, peanut, and peanut butter consumption and the risk of pancreatic cancer in the Netherlands Cohort Study

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Abstract

Background: Nut intake has been associated with decreased cancer-related mortality, but few studies have examined the potential of nuts in the chemoprevention of pancreatic cancer. We prospectively investigated the association of total nut, tree nut, peanut, and peanut butter consumption with pancreatic cancer risk.

Methods: In the Netherlands Cohort Study, 120,852 men and women completed a baseline questionnaire, including a food frequency questionnaire, in 1986. After 20.3 years of follow-up, 583 incident pancreatic cancer cases, including 349 microscopically confirmed pancreatic cancer (MCPC) cases, were included in multivariable case-cohort analyses.

Results: Increased total nut consumption was associated with a nonsignificantly decreased MCPC risk in men (HR (95% CI) for 10+ g/day vs. nonconsumers = 0.72 (0.47-1.11), P_{trend} = 0.163). No clear association was found in women. For tree nut and peanut consumption, nonsignificant inverse associations were observed in men. In women, no or unclear associations were found for tree nut and peanut consumption. Peanut butter intake was related to a significantly reduced risk of MCPC in men (HR (95% CI) for 5+ g/day vs. nonconsumers = 0.53 (0.28-1.00), P_{trend} = 0.047), but this relation was not clear in women. Evidence for a nonlinear dose-response relation with MCPC was found for tree nut intake only. The associations were weaker when looking at total pancreatic cancer.

Conclusion: Our results suggest that nuts and peanut butter might reduce pancreatic cancer risk in men. In women, no or unclear associations were found.

Impact: Nut consumption might reduce the risk of pancreatic cancer in men.

Introduction

In 2012, pancreatic cancer was the seventh leading cause of cancer-related mortality worldwide, whereas its incidence ranked 12th of all cancers (1). Overall 5-year survival rates are estimated to be 8%, but only 2% for patients with metastasized disease (2). These low survival rates are due to the fact that the early disease stages usually are asymptomatic. Consequently, patients are often diagnosed when at an advanced stage of pancreatic cancer when curative surgical resection is not always possible (2-4). Moreover, no screening tests are available currently (4). Therefore, preventive strategies are urgently needed.

In literature, several modifiable lifestyle and dietary factors, such as smoking, obesity, and alcohol and red meat consumption, are suggested to increase pancreatic cancer risk, whereas a diet rich in vegetables, fruits, and whole grains might contribute to its prevention (5, 6). Nuts represent another food group that has been investigated for its potential cancer-chemopreventive activities, because recent meta-analyses have shown that increased nut consumption might decrease cancer risk and cancer-related mortality (7-10).

Nuts contain numerous bioactive compounds such as vitamin B6 and E, folate, selenium, fiber, mono- and polyunsaturated fatty acids, and many polyphenols (11, 12). Although the exact biological mechanism by which nuts might reduce pancreatic cancer risk has yet to be elucidated, possible mechanisms suggested in literature mainly relate to their antioxidant, anti-inflammatory, and immune modulating activities (6, 13-15). Moreover, bioactive compounds in nuts might contribute to normal cell differentiation and DNA repair mechanisms, reduced tumor initiation and promotion, reduced angiogenesis, and induced apoptosis (6, 13-15). In addition, nut consumption might beneficially affect obesity, type 2 diabetes mellitus, and pancreatitis, which are well known risk factors for pancreatic cancer (5, 6, 15).

To our knowledge, only two studies have investigated the association between nut consumption and pancreatic cancer risk in humans (16, 17). In a case-control study, no statistically significant relation was found between the consumption of “nuts and tasty snacks” and pancreatic cancer risk (16). Because nuts and tasty snacks were analyzed together as exposure variable, it is not possible to draw conclusions from this study for nut consumption alone. In a prospective cohort study, a significant inverse association was found between nut consumption frequency and pancreatic cancer risk (17). Nevertheless, this study was limited to women. Furthermore, little is known about whether the relation with pancreatic cancer risk differs between tree nuts, peanuts, and peanut butter.

In the present study, we investigated the association of total nut, tree nut, peanut, and peanut butter intake with the risk of pancreatic cancer in both men and women in the Netherlands Cohort Study on Diet and Cancer (NLCS).

Materials and Methods

Study design and population

The current study was performed within the NLCS, a prospective cohort study in the Netherlands initiated on September 17, 1986, to assess the relation between diet and cancer. Details of this study are reported elsewhere (18). In short, 120,852 males and females aged 55-69 years from 204 Dutch municipalities with computerized population registries were included. For efficiency reasons, a case-cohort design was used for data processing and analyses, by randomly sampling 5,000 participants from the total cohort at baseline to create a subcohort. Cancer cases were obtained from the total cohort, whereas person-years at risk were calculated in the subcohort as an estimation of the total person-years at risk in the entire cohort.

At baseline, participants completed a self-administered 11-page questionnaire, including a 150-item semiquantitative food frequency questionnaire (FFQ), on cancer risk factors. By filling in and returning the baseline questionnaire, participants agreed to participate in the NLCS. The entire cohort was followed up for cancer incidence during the subsequent 20.3 years (baseline until December 31, 2006) through annual record linkage to the Netherlands Cancer Registry and the Dutch National Database of Pathology Reports (PALGA) (19). Cancer incidence follow-up is estimated to be at least 96% complete (20). Data on vital status of subcohort members were 100% complete after 20.3 years of follow-up. The institutional review boards of the Netherlands Organization for Applied Scientific Research TNO (Zeist) and Maastricht University (Maastricht) approved the NLCS. The NLCS was conducted in accordance with the Declaration of Helsinki.

The study population used in this analysis consisted of all subcohort members and incident pancreatic cancer cases (ICD-O-3 code C25), except for endocrine subtypes (ICD-O-3 code C25.4), diagnosed during the follow-up period. Endocrine subtypes were excluded because of their different etiology and rarity. Cases were diagnosed by microscopic confirmation or physician diagnosis, which was made based on either clinical symptoms, physical examination, or imaging results.

Subcohort members and incident exocrine pancreatic cancer cases from the entire cohort were included if they had no prevalent cancer at baseline other than skin cancer. This resulted in 4,774 subcohort members and 763 pancreatic cancer cases, including 454 microscopically confirmed pancreatic cancer (MCPC) cases. Participants were then excluded if they had left more than 60 items or at least one item block of the FFQ blank, or if they had eaten less than 35 food items at least once per month. Subjects with missing data on confounding variables were excluded as well. Figure 1 presents a flow diagram of the number of subcohort members and cases on whom the analysis was based. In total, 3,759 subcohort members (78.7% of 4,774) and 583 pancreatic cancer cases (76.4% of 763), including 349 MCPC cases (76.9% of 454), were available for analysis.

Exposure measurement

The baseline questionnaire measured smoking habits, physical activity, anthropometry, disease history, dietary intake, and other cancer risk factors. The FFQ assessed information about habitual diet in the preceding year, including the consumption of 'peanuts', 'other nuts, mixed nuts' (tree nuts) and 'peanut butter'. The consumption frequency could range from 'never or less than 1x/month' to '6-7x/week'. In addition, participants could fill in the number of standard portion sizes they consumed per intake. For tree nuts and peanuts, a standard portion size was 28 grams. A standard portion size of peanut butter, a particularly popular spread in the Netherlands, was 15 grams per slice of bread. Consumption frequencies and portion sizes were multiplied to calculate mean daily intakes in grams. Total nut consumption was calculated as the sum of peanuts and tree nuts. To prevent observer bias, NLCS-personnel was blinded to the case/subcohort status of the participants during the entry, coding, and interpretation of the questionnaire data.

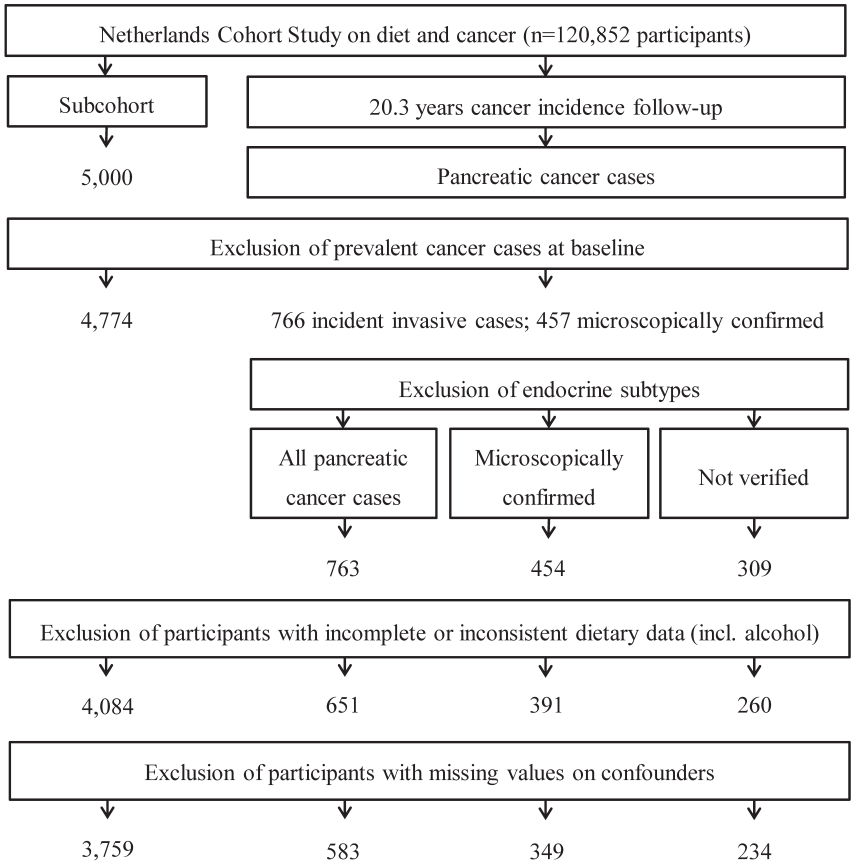

Figure 1. Flow diagram of the number of subcohort members and cases on whom the analyses were based

Statistical analysis

The relation between nut and peanut butter consumption and pancreatic cancer risk was investigated with Cox proportional hazards models to estimate age- and sex-adjusted and multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI). Person-years at risk were calculated in the subcohort from the date of entry in the cohort (September 17, 1986) until pancreatic cancer diagnosis or censoring. Participants were censored in the case of loss to follow-up, death, migration, or end of follow-up (December 31, 2006), whichever occurred first.

To compare energy-adjustment methods, multivariable-adjusted models including daily total energy intake were compared to nutrient density models. Because the results of these models were similar, we present only those obtained from the first method.

Confounders were selected on the basis of literature and earlier pancreatic cancer analyses in the NLCS (21-29), and were defined as those variables that were related to both the exposure and outcome. Predefined confounders, which were included in the final model irrespective of their effect on the estimates, were: age (years; continuous); sex (men/women; in the analyses for the total population); cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered) and duration (years; continuous, centered)); body mass index (BMI) (kg/m^2; continuous); family history of pancreatic cancer (no/yes); history of diabetes (no/yes); educational level (primary school or lower vocational education (low)/ secondary school or medium vocational education (medium)/university or higher vocational education (high)); total energy intake (kcal/day; continuous) and alcohol consumption (g/day; continuous). Potential confounders considered were height, nonoccupational physical activity, history of gallstones, cholecystectomy, gastric ulcers, hypertension, and hepatitis, intake of fruit, vegetables, red meat, and coffee, and nutritional supplement use. A variable was regarded as a confounder if it changed the HR with at least 10% when using a backward stepwise selection procedure. On the basis of this procedure, only the predefined confounders were included in the final multivariable-adjusted model.

All analyses were performed for males and females separately and for the total population, for total pancreatic cancer (non-MCPC and MCPC combined) and for MCPC alone. The restriction to MCPC cases was performed to obtain a higher degree of diagnostic certainty, because cases without histological confirmation may reflect other types or nonpancreatic cancers (29).

Total nut, tree nut, peanut, and peanut butter intakes were analyzed separately on a categorical and continuous scale. For the categorical analyses, nut and peanut butter consumption were categorized as follows: 0, 0.1-<5, 5-<10, 10+ g/day for total nuts and peanuts and 0, 0.1-<5, 5+ g/day for tree nuts and peanut butter, because of the smaller numbers of cases in the higher intake categories. The lowest intake category was regarded as the reference group. Linear trends between nut and peanut butter consumption categories and pancreatic cancer risk were evaluated with Wald tests, after fitting median values of nut consumption per intake category as continuous terms in the regression models. Median

values were based on the distribution of the variables in the subcohort. For continuous analyses, an increment of five grams per day was chosen.

Standard errors were calculated using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort (30). The validity of the proportional hazard assumption was checked for each covariate based on scaled Schoenfeld residuals (31), by visual inspection of log-minus-log survival plots, and by including time-covariate interactions into the models. The statistical significance of these interaction terms was evaluated with the Wald test. No violations were found for the exposure variables. In cases where the proportional hazards assumption was violated for confounders, time-varying covariates were kept in the model.

To further investigate the dose-response relations between nut consumption and pancreatic cancer risk, restricted cubic splines with three fixed knots (0, 5, and 10 g/day) were used to graphically present the dose-response curves without making a priori assumptions about their shapes. Wald tests were performed to evaluate the linearity of these relations.

Possible interactions with other pancreatic cancer risk factors were evaluated by analyzing total nut consumption and pancreatic cancer risk in strata of the following factors: baseline BMI (18.5-<25/≥25 kg/m^2), smoking status (never/former/current), alcohol consumption (0/0.1-<15/≥15 g/day), and educational level (low/medium/high). Interactions with these factors were tested by including cross-product terms in the Cox regression models and performing Wald tests.

All *P*-values were two-sided and were considered statistically significant if smaller than 5%. Analyses were performed using Stata software (Version 14.0; StataCorp).

Sensitivity analysis

As additional analyses, associations of tree nut, peanut, and peanut butter consumption with pancreatic cancer risk were mutually adjusted. The analyses were also repeated with nonconsumers of both nuts and peanut butter as the reference category. To check for potential reversed causation, median nut consumption of cases diagnosed in the first two years of follow-up was compared with the median nut intake of cases diagnosed later in time. Subsequently, Kruskal-Wallis tests were performed to test whether these were significantly different. In addition, a subgroup analysis was performed in which pancreatic cancer cases diagnosed in the first two years of follow-up were excluded. In another subgroup analysis, respondents with diabetes were excluded. Moreover, analyses of peanut butter consumption were repeated, restricted to respondents who had stated having had a constant peanut butter intake during the five years before baseline. Unfortunately, these data were unavailable for total nut, tree nut, and peanut consumption.

Additional adjustment was performed for adherence to the Mediterranean diet as measured with the alternate Mediterranean diet (aMed) (32) score. Because nuts comprise one of the components of the aMed score and because alcohol consumption is positively associated

with pancreatic cancer risk, an adapted version was used (excluding nuts and alcohol), which ranged from 0 (no adherence) to 7 (maximal adherence) (33).

To determine the sensitivity of the nonparametric response curves to assumptions regarding the number and placement of knots, detailed cubic spline regression analyses were performed. In these, the performance of three additional models ((i) three fixed knots: 0, 1, and 5 g/d, (ii) percentiles: 10^{th}, 50^{th}, and 90^{th} percentiles, and (iii) four fixed knots: 0, 1, 5, and 10 g/d) were compared with the model with three fixed knots (0, 5, and 10 g/d) using the Akaike Information Criterium (AIC) (34).

An array approach sensitivity analysis (35) was performed to determine how strong and imbalanced unmeasured confounders would have to be to alter the association between total nut consumption and pancreatic cancer risk.

Results

Mean total nut consumption (SD) in the subcohort was 8.0 (14.1) g/day in men and 4.3 (8.4) g/day in women. For tree nut, peanut, and peanut butter, these values were 1.0 (3.4), 7.0 (13.3), and 1.4 (4.2) in men and 1.1 (3.9), 3.3 (6.9), and 1.2 (3.6) in women, respectively. The percentages of men in the subcohort, among all pancreatic cancer cases, and among MCPC cases were 49.2%, 51.1%, and 53.9%, respectively. Table 1 presents baseline characteristics, stratified by gender. Participants with a higher nut intake were younger, higher educated, less frequently hypertensive, had a higher total energy intake, consumed more alcohol and fruit, and used nutritional supplements more often. Females who consumed more nuts were leaner and more often ever smokers, consumed more vegetables and red meat, less often reported a history of gallstones or cholecystectomy, and those in the highest consumption category were less likely to be diabetic. Males with a higher nut intake were less likely to report a history of gastric ulcers or a positive family history of pancreatic cancer.

Table 2 shows the age- and sex-adjusted and multivariable-adjusted HRs for total pancreatic cancer according to total nut, tree nut, peanut, and peanut butter consumption in men and women separately and in the total population. In multivariable-adjusted analyses, increasing total nut intake was associated with a nonstatistically significant decreasing risk of total pancreatic cancer in men (HR (95% CI) for 10+ g/day vs. nonconsumers = 0.71 (0.50-1.03), P_{trend} = 0.097). No association was found in women and a nonstatistically significant inverse association in the total population. For tree nuts and peanuts, also nonstatistically significant decreasing risks were found with increasing intake in men (P_{trend} = 0.106 and 0.293, respectively), and a statistically significant reduced risk for the category of 0.1-<5 g tree nut consumption/day compared to nonconsumers (HR (95% CI) = 0.68 (0.48-0.95)). In women, tree nut consumption was not associated with total pancreatic cancer risk. When looking at the results of both the categorical and continuous analyses, the relation for peanut intake was not clear in women. In the total population, nonstatistically significant inverse associations were found for tree nut and peanut intake. Because the number of cases in the

Table 1. Baseline characteristics (mean (SD) or percent) according to total nut intake in male and female subcohort members

	Men				Women			
	Total nut intake (g/day) (median)				Total nut intake (g/day) (median)			
	0 (0.0)	0.1-<5 (2.5)	5-<10 (8.5)	10+ (21.4)	0 (0.0)	0.1-<5 (2.1)	5-<10 (7.8)	10+ (15.7)
n[a]	584	596	236	434	773	692	214	230
Age (y)	61.8 (4.4)	61.2 (4.1)	60.8 (4.3)	60.5 (4.0)	62.2 (4.3)	61.1 (4.2)	60.1 (4.0)	60.7 (3.9)
BMI (kg/m^2)	25.0 (2.7)	24.8 (2.4)	24.9 (2.6)	25.0 (2.5)	25.3 (3.8)	25.1 (3.4)	24.3 (3.2)	24.5 (3.3)
Height (cm)	175.7 (6.6)	176.9 (6.4)	177.5 (6.5)	176.8 (6.8)	165.0 (6.4)	165.5 (5.8)	165.6 (6.0)	165.9 (6.2)
Ever cigarette smokers (%)	86.8	84.7	85.2	88.5	37.8	39.5	48.1	49.1
University or higher vocational education (%)	15.8	19.1	24.6	25.1	6.1	10.8	14.0	12.6
Nonoccupational physical activity (min/d)	83.0 (72.2)	82.1 (66.6)	71.1 (56.8)	82.3 (66.8)	63.4 (54.1)	67.2 (48.6)	72.5 (55.6)	61.0 (37.1)
Family history of pancreatic cancer (%)	1.5	0.3	0.9	0.7	1.0	1.0	0.5	1.3
History of diabetes (%)	4.5	2.4	3.8	2.8	3.5	3.8	3.3	0.9
History of gallstones (%)	5.5	4.9	5.1	4.8	15.8	14.2	12.2	9.1
History of cholecystectomy (%)	4.3	4.4	4.7	4.4	15.4	12.6	9.8	10.0
History of gastric ulcer (%)	13.9	11.2	11.0	11.1	5.8	4.2	1.4	3.5
History of hypertension (%)	25.3	24.5	24.2	22.8	32.3	27.0	23.8	25.2
History of hepatitis (%)	9.3	12.3	13.1	11.3	12.9	16.2	17.3	13.5
Food intake								
Energy (kcal/d)	2,100 (528)	2,080 (462)	2,212 (469)	2,353 (466)	1,594 (375)	1,672 (370)	1,785 (411)	1,922 (360)
Alcohol (g/d)	13.0 (16.7)	13.4 (16.4)	16.7 (18.5)	19.1 (16.8)	4.9 (9.4)	5.9 (8.8)	7.1 (9.4)	8.8 (11.1)
Fruit (g/d)	148.4 (114.2)	155.0 (102.5)	166.3 (134.3)	163.0 (120.5)	184.4 (120.6)	197.3 (112.5)	206.7 (119.3)	210.5 (123.8)
Vegetables (g/d)	187.1 (81.5)	187.3 (70.4)	188.0 (75.6)	186.0 (73.5)	182.9 (74.5)	195.1 (76.3)	200.5 (73.8)	204.6 (72.7)
Red meat (g/d)	94.1 (45.8)	91.9 (39.0)	92.9 (35.9)	95.4 (41.7)	79.8 (39.5)	80.6 (35.4)	81.0 (37.8)	83.5 (38.8)
Coffee (g/d)	572.1 (333.4)	563.8 (263.5)	549.5 (256.7)	586.6 (252.5)	494.1 (254.9)	506.1 (238.7)	502.2 (246.1)	500.4 (213.5)
Nutritional supplement user (%)	21.9	22.5	27.1	25.4	34.2	36.9	38.8	44.8

[a] Number of subcohort members excluding participants with incomplete or inconsistent dietary data (including alcohol consumption) or missing values on predefined confounding variables.

Table 2. Age- and sex-adjusted and multivariable-adjusted HRs (and 95% CIs) for total pancreatic cancer according to nut consumption; NLCS, 1986-2006

	Median intake[a]		Men				Women				Total population	
	Men	Women	Person-years	Cases	Age-adjusted HR (95% CI)	Multivariable-adjusted HR[b] (95% CI)	Person-years	Cases	Age-adjusted HR (95% CI)	Multivariable-adjusted HR[b] (95% CI)	Age- and sex-adjusted HR (95% CI)	Multivariable-adjusted HR[c] (95% CI)
Total nuts (g/day)												
0	0.0	0.0	8,888	108	1.00 (reference)	1.00 (reference)	13,527	115	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	2.5	2.1	9,675	94	0.80 (0.59-1.09)	0.85 (0.62-1.17)	12,389	108	1.10 (0.83-1.45)	1.11 (0.83-1.49)	0.94 (0.77-1.16)	0.98 (0.79-1.22)
5-<10	8.5	7.8	3,951	37	0.78 (0.52-1.17)	0.78 (0.50-1.19)	3,883	26	0.91 (0.57-1.44)	0.89 (0.54-1.44)	0.84 (0.62-1.15)	0.85 (0.62-1.16)
10+	21.4	15.7	7,124	59	0.71 (0.50-1.00)	0.71 (0.50-1.03)	4,280	36	1.09 (0.72-1.63)	0.98 (0.63-1.50)	0.85 (0.65-1.11)	0.84 (0.63-1.11)
P_{trend}					0.097	0.097			0.912	0.679	0.223	0.165
Continuous, per 5 g/day increment					0.96 (0.91-1.01)	0.95 (0.90-1.01)			1.00 (0.93-1.07)	0.97 (0.90-1.06)	0.97 (0.93-1.01)	0.96 (0.92-1.01)
Tree nuts (g/day)												
0	0.0	0.0	21,393	239	1.00 (reference)	1.00 (reference)	23,847	204	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	1.6	1.6	6,704	48	0.64 (0.46-0.89)	0.68 (0.48-0.95)	8,365	71	1.03 (0.77-1.38)	1.02 (0.74-1.39)	0.83 (0.66-1.03)	0.86 (0.69-1.08)
5+	8.6	8.9	1,541	11	0.64 (0.34-1.21)	0.66 (0.34-1.26)	1,866	10	0.67 (0.34-1.29)	0.63 (0.32-1.24)	0.65 (0.41-1.03)	0.65 (0.41-1.04)
P_{trend}					0.077	0.106			0.262	0.206	0.038	0.051
Continuous, per 5 g/day increment					0.85 (0.62-1.17)	0.86 (0.63-1.18)			0.98 (0.77-1.24)	0.96 (0.75-1.24)	0.92 (0.75-1.13)	0.93 (0.75-1.14)
Peanuts (g/day)												
0	0.0	0.0	9,995	114	1.00 (reference)	1.00 (reference)	15,936	136	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	2.5	2.1	10,542	104	0.88 (0.66-1.18)	0.92 (0.68-1.24)	12,635	102	1.03 (0.78-1.35)	1.05 (0.79-1.40)	0.96 (0.78-1.17)	0.99 (0.80-1.22)
5-<10	8.5	8.5	3,285	29	0.81 (0.52-1.25)	0.77 (0.49-1.21)	2,556	24	1.26 (0.78-2.04)	1.11 (0.67-1.84)	0.99 (0.71-1.37)	0.94 (0.67-1.31)
10+	21.4	17.1	5,816	51	0.81 (0.56-1.16)	0.82 (0.56-1.19)	2,951	23	1.01 (0.62-1.63)	0.94 (0.57-1.55)	0.89 (0.67-1.19)	0.88 (0.65-1.19)
P_{trend}					0.303	0.293			0.742	0.885	0.491	0.372
Continuous, per 5 g/day increment					0.96 (0.91-1.02)	0.96 (0.91-1.02)			1.00 (0.92-1.08)	0.97 (0.88-1.06)	0.97 (0.93-1.02)	0.96 (0.92-1.01)
Peanut butter (g/day)												
0	0.0	0.0	21,202	216	1.00 (reference)	1.00 (reference)	24,879	209	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	1.2	1.2	5,164	61	1.21 (0.89-1.66)	1.24 (0.89-1.72)	5,967	54	1.13 (0.82-1.56)	1.11 (0.79-1.55)	1.17 (0.94-1.47)	1.19 (0.95-1.51)
5+	9.6	6.9	3,272	21	0.66 (0.41-1.06)	0.66 (0.41-1.08)	3,232	22	0.85 (0.53-1.37)	0.86 (0.53-1.39)	0.74 (0.53-1.04)	0.75 (0.53-1.05)
P_{trend}					0.079	0.095			0.553	0.566	0.083	0.095
Continuous, per 5 g/day increment					0.93 (0.77-1.11)	0.93 (0.77-1.13)			0.91 (0.74-1.12)	0.90 (0.73-1.11)	0.92 (0.80-1.06)	0.92 (0.80-1.06)

[a] Median intake in the subcohort.

[b] Adjusted for age (years; continuous); cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered) and duration (years; continuous, centered)); BMI (kg/m^2; continuous); family history of pancreatic cancer (no/yes); history of diabetes (no/yes); educational level (low/medium/high); total energy intake (kcal/day; continuous) and alcohol consumption (g/day; continuous).

[c] Adjusted for [b] and sex (male/female).

highest intake category of tree nuts was small in both sexes, categories were merged into consumers (0.1+ g/day) and nonconsumers. The HR (95% CI) for total pancreatic cancer for tree nut consumers versus nonconsumers was 0.67 (0.49-0.92) in men and 0.95 (0.70-1.28) in women. Associations for peanut butter intake were not clear.

Table 3 presents the HRs for MCPC according to nut and peanut butter consumption. Overall, associations with nut consumption categories were stronger when cases were restricted to MCPC cases, and some became significant in men. Increased total nut consumption was associated with a nonsignificantly decreased MCPC risk in men (P_{trend} = 0.163). Although intake of 5-<10 g total nuts/day was related to a significantly reduced risk in men (HR (95% CI) = 0.56 (0.32-0.99)), intake of 10+ g/day was not. No clear association was found for total nut consumption in women, and a nonsignificant inverse trend in the total population. For tree nut and peanut consumption, nonsignificant inverse associations were observed in men (P_{trend} = 0.214 and 0.407, respectively), although peanut consumption of 5-<10 g/day compared to nonconsumption was associated with a significantly reduced risk (HR (95% CI) = 0.44 (0.23-0.85)). In women, no association was found for tree nut consumption and no clear association for peanut consumption. In the total population, nonsignificant inverse trends were found for both tree nuts and peanuts. When comparing tree nut consumers (0.1+ g/day) to nonconsumers, the HR (95% CI) for MCPC was 0.68 (0.47-1.00) in men and 0.89 (0.60-1.33) in women. Peanut butter intake was related to a significantly reduced MCPC risk in men (HR (95% CI) for 5+ g/day vs. nonconsumers = 0.53 (0.28-1.00), P_{trend} = 0.047), but this relations was not clear in women and in the total population.

Because of the higher diagnostic certainty and the, in general, stronger associations in MCPC cases, subsequent analyses were performed using this case definition.

No significant interaction was observed between gender and total nut intake (Table 4, $P_{interaction}$ = 0.377). For the nut types separately, no significant interactions with gender were found either. For this reason, and to increase statistical power, men and women were combined in subsequent analyses. In restricted cubic spline analyses, evidence for a non-linear dose-response relation was found for tree nut intake ($P_{nonlinearity}$ = 0.005), but not for total nut, peanut, and peanut butter intake ($P_{nonlinearity}$ = 0.669, 0.598, and 0.778, respectively). In both sexes combined, MCPC risk was significantly decreasing with increasing tree nut intake from 0.1 to 7.5 g/day, but this association weakened somewhat for intakes above 7.5 g/day. Increasing total nut, peanut, and peanut butter intake were associated with a nonsignificantly decreasing MCPC risk. In Figure 2, nonparametric regression curves are shown.

Table 4 presents the associations between total nut consumption and MCPC risk in strata of potential effect modifiers. To increase statistical power, the two highest intake categories were merged. No significant interactions were found for any of the potential effect modifiers: *P*-values for interaction were ≥0.082. In most subgroups, associations were inverse or not clear, although significant inverse trends were found in men, in participants with a normal BMI (18.5-<25 kg/m^2), and in former smokers.

Table 3. Age-and sex-adjusted and multivariable-adjusted HRs (and 95% CIs) for microscopically confirmed pancreatic cancer according to nut consumption; NLCS, 1986-2006

	Median intake[a]		Men				Women				Total population	
	Men	Women	Person-years	Cases	Age-adjusted HR (95% CI)	Multivariable-adjusted HR[b] (95% CI)	Person-years	Cases	Age-adjusted HR (95% CI)	Multivariable-adjusted HR[b] (95% CI)	Age- and sex-adjusted HR (95% CI)	Multivariable-adjusted HR[c] (95% CI)
Total nuts (g/day)												
0	0.0	0.0	8,888	68	1.00 (reference)	1.00 (reference)	13,529	60	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	2.5	2.1	9,675	62	0.84 (0.58-1.21)	0.86 (0.59-1.24)	12,393	67	1.24 (0.86-1.78)	1.23 (0.84-1.79)	1.02 (0.79-1.32)	1.03 (0.79-1.35)
5-<10	8.5	7.8	3,951	18	0.60 (0.35-1.03)	0.56 (0.32-0.99)	3,883	14	0.84 (0.46-1.55)	0.81 (0.43-1.54)	0.71 (0.47-1.06)	0.68 (0.45-1.04)
10+	21.4	15.7	7,124	40	0.75 (0.50-1.13)	0.72 (0.47-1.11)	4,280	20	1.08 (0.64-1.82)	0.99 (0.56-1.73)	0.88 (0.63-1.22)	0.85 (0.60-1.19)
P_{trend}					0.218	0.163			0.848	0.602	0.270	0.196
Continuous, per 5 g/day increment					0.96 (0.90-1.03)	0.95 (0.89-1.03)			0.98 (0.88-1.08)	0.96 (0.86-1.07)	0.96 (0.91-1.02)	0.96 (0.90-1.02)
Tree nuts (g/day)												
0	0.0	0.0	21,394	150	1.00 (reference)	1.00 (reference)	23,851	116	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	1.6	1.6	6,704	31	0.66 (0.44-0.99)	0.68 (0.45-1.03)	8,367	41	1.02 (0.70-1.47)	1.00 (0.67-1.49)	0.83 (0.63-1.08)	0.85 (0.64-1.13)
5+	8.6	8.9	1,542	7	0.65 (0.30-1.43)	0.68 (0.31-1.50)	1,866	4	0.45 (0.16-1.23)	0.42 (0.15-1.20)	0.56 (0.30-1.04)	0.56 (0.30-1.06)
P_{trend}					0.169	0.214			0.116	0.105	0.043	0.056
Continuous, per 5 g/day increment					0.91 (0.63-1.31)	0.92 (0.64-1.33)			0.96 (0.65-1.42)	0.95 (0.62-1.44)	0.93 (0.70-1.24)	0.94 (0.71-1.25)
Peanuts (g/day)												
0	0.0	0.0	9,995	72	1.00 (reference)	1.00 (reference)	15,938	70	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	2.5	2.1	10,542	69	0.92 (0.65-1.30)	0.92 (0.64-1.32)	12,639	62	1.14 (0.80-1.64)	1.15 (0.80-1.67)	1.03 (0.80-1.32)	1.04 (0.80-1.35)
5-<10	8.5	8.5	3,285	11	0.48 (0.25-0.92)	0.44 (0.23-0.85)	2,556	16	1.49 (0.83-2.65)	1.35 (0.73-2.47)	0.83 (0.53-1.28)	0.77 (0.50-1.21)
10+	21.4	17.1	5,816	36	0.89 (0.58-1.36)	0.85 (0.55-1.33)	2,951	13	1.03 (0.56-1.91)	0.98 (0.51-1.88)	0.97 (0.68-1.37)	0.94 (0.65-1.35)
P_{trend}					0.508	0.407			0.647	0.894	0.700	0.550
Continuous, per 5 g/day increment					0.96 (0.90-1.03)	0.95 (0.89-1.02)			0.98 (0.88-1.08)	0.95 (0.85-1.06)	0.96 (0.91-1.02)	0.95 (0.90-1.01)
Peanut butter (g/day)												
0	0.0	0.0	21,203	142	1.00 (reference)	1.00 (reference)	24,883	112	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	1.2	1.2	5,164	35	1.04 (0.70-1.53)	1.05 (0.70-1.57)	5,970	34	1.28 (0.86-1.91)	1.27 (0.84-1.93)	1.14 (0.86-1.51)	1.16 (0.87-1.55)
5+	9.6	6.9	3,272	11	0.51 (0.27-0.97)	0.53 (0.28-1.00)	3,232	15	1.04 (0.59-1.83)	1.05 (0.58-1.88)	0.73 (0.48-1.11)	0.73 (0.48-1.12)
P_{trend}					0.037	0.047			0.807	0.823	0.139	0.141
Continuous, per 5 g/day increment					0.86 (0.67-1.11)	0.87 (0.68-1.12)			0.96 (0.77-1.19)	0.94 (0.75-1.17)	0.90 (0.77-1.07)	0.90 (0.76-1.06)

[a] Median intake in the subcohort.

[b] Adjusted for age (years; continuous); cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered) and duration (years; continuous, centered)); BMI (kg/m^2; continuous); family history of pancreatic cancer (no/yes); history of diabetes (no/yes); educational level (low/medium/high); total energy intake (kcal/day; continuous) and alcohol consumption (g/day; continuous).

[c] Adjusted for [b] and sex (male/female).

Mutual adjustment for tree nut, peanut, and peanut butter did not importantly alter the results for the associations with total pancreatic cancer and MCPC, and neither did choosing nonconsumers of both nuts and peanut butter as the reference category. Median total nut, tree nut, peanut, and peanut butter intake of MCPC cases diagnosed in the first two years of follow-up were similar to the intakes of MCPC cases diagnosed later in time (P ≥0.233). Exclusion of patients diagnosed during the first two years of follow-up did not importantly alter the results. Excluding respondents with diabetes at baseline gave comparable results as well. Repeating the analyses of peanut butter consumption, restricted to participants who had stated having had a constant intake of peanut butter during the five years before baseline, gave similar results as when all participants were included. Additional adjustment for the adapted aMed score did not essentially alter the results.

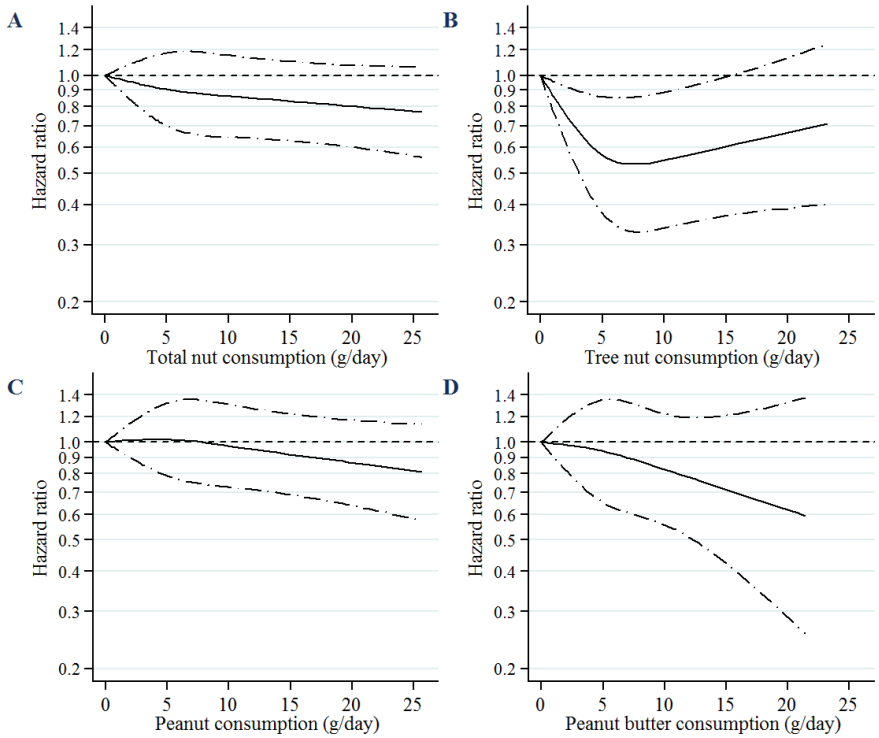

Figure 2. Nonparametric regression curves for the associations between nut and peanut butter consumption and microscopically confirmed pancreatic cancer. It presents the nonparametric regression curves for the associations between (**A**) total nut, (**B**) tree nut, (**C**) peanut, and (**D**) peanut butter consumption and microscopically confirmed pancreatic cancer risk. Solid lines represent point estimates, dashed lines represent 95% CIs. *P*-values for nonlinearity were 0.669 for total nuts, 0.005 for tree nuts, 0.598 for peanuts, and 0.778 for peanut butter. Multivariable HRs were calculated using restricted cubic splines with three fixed knots (consumption of 0, 5, and 10 g/day), adjusted for age (years; continuous); sex (male/female); cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered) and duration (years; continuous, centered)); BMI (kg/m^2; continuous); family history of pancreatic cancer (no/yes); history of diabetes (no/yes); educational level (low/ medium/ high); total energy intake (kcal/day; continuous) and alcohol consumption (g/day; continuous).

Table 4. HRs (95% CIs) for microscopically confirmed pancreatic cancer according to total nut intake in men and women combined in subgroups, in multivariable-adjusted analyses[a], the Netherlands Cohort Study, 1986-2006

	Total nut consumption (g/day) (median[b])			P_{trend}	$P_{interaction}$
	0 g/day (0.0)	0.1-<5 g/day (2.5)	5+ g/day (11.5)		
Overall					
Cases/person-time at risk (years)	128/22,417	129/22,068	92/19,238		
HR (95% CI)	1.00 (reference)	1.03 (0.79-1.35)	0.78 (0.58-1.05)	0.060	
Sex					
Males					
Cases/person-time at risk (years)	68/8,888	62/9,675	58/11,075		
HR (95% CI)	1.00 (reference)	0.86 (0.59-1.24)	0.66 (0.45-0.98)	0.043	0.377
Females					
Cases/person-time at risk (years)	60/13,529	67/12,393	34/8,163		
HR (95% CI)	1.00 (reference)	1.23 (0.84-1.79)	0.90 (0.56-1.45)	0.451	
Total population					
BMI					
18.5-<25 kg/m^2					
Cases/person-time at risk (years)	64/11,288	56/12,036	39/11,210		
HR (95% CI)	1.00 (reference)	0.85 (0.57-1.26)	0.60 (0.39-0.94)	0.026	0.082
≥25 kg/m^2					
Cases/person-time at risk (years)	63/10,837	73/9,904	53/7,924		
HR (95% CI)	1.00 (reference)	1.24 (0.86-1.78)	0.97 (0.64-1.47)	0.597	
Cigarette smoking					
Never					
Cases/person-time at risk (years)	37/9,919	40/9,208	23/5,778		
HR (95% CI)	1.00 (reference)	1.12 (0.70-1.79)	0.92 (0.51-1.64)	0.662	0.260
Former					
Cases/person-time at risk (years)	50/6,461	46/7,954	35/8,511		
HR (95% CI)	1.00 (reference)	0.82 (0.52-1.27)	0.54 (0.34-0.87)	0.012	
Current					
Cases/person-time at risk (years)	41/6,037	43/4,907	34/4,948		
HR (95% CI)	1.00 (reference)	1.19 (0.73-1.92)	1.04 (0.62-1.76)	0.957	
Alcohol consumption					
0 g/day					
Cases/person-time at risk (years)	34/7,916	25/4,691	8/2,259		
HR (95% CI)	1.00 (reference)	1.26 (0.72-2.22)	0.69 (0.29-1.61)	0.357	0.757
0.1-<15 g/day					
Cases/person-time at risk (years)	63/10,028	69/12,731	44/10,348		
HR (95% CI)	1.00 (reference)	0.92 (0.64-1.33)	0.74 (0.48-1.13)	0.156	
≥15 g/day					
Cases/person-time at risk (years)	31/4,473	35/4,647	40/6,631		
HR (95% CI)	1.00 (reference)	1.12 (0.64-1.94)	0.83 (0.48-1.45)	0.335	
Educational level					
Low					
Cases/person-time at risk (years)	71/12,894	55/10,788	38/7,450		
HR (95% CI)	1.00 (reference)	1.00 (0.68-1.46)	0.87 (0.56-1.35)	0.500	0.716
Medium					
Cases/person-time at risk (years)	40/7,213	52/8,018	38/7,866		
HR (95% CI)	1.00 (reference)	1.16 (0.74-1.82)	0.83 (0.51-1.34)	0.246	
High					
Cases/person-time at risk (years)	17/2,310	22/3,263	16/3,921		
HR (95% CI)	1.00 (reference)	0.87 (0.43-1.78)	0.55 (0.25-1.21)	0.123	

[a] Adjusted for age (years; continuous); sex (male/female); cigarette smoking (status (never/former/current), frequency (n/day; continuous, centered) and duration (years; continuous, centered)); BMI (kg/m^2; continuous); family history of pancreatic cancer (no/yes); history of diabetes (no/yes); educational level (low/medium/ high); total energy intake (kcal/day; continuous) and alcohol consumption (g/day; continuous).
[b] Median intake in the subcohort.

The sensitivity analyses regarding the assumptions in restricted cubic spline analyses showed that including an additional knot or using different knot positions did not improve the performance of the model with three fixed knots at 0, 5, and 10 g nut intake/day, as measured with the AIC score.

For the array approach sensitivity analysis, the observed HR for MCPC was set at 0.78 (HR for 5+ g total nut consumption/day vs. nonconsumers in males and females combined, Table 4) and the prevalence of the unmeasured confounder among nonconsumers at 0.3. The analysis showed that the true, adjusted HR would exceed the value of one if the prevalence of the unmeasured confounder in the category of 5+ g total nut consumption/day were ≤0.1 and the HR for MCPC according to the unmeasured confounder ≥2.0, or if the prevalence were ≥0.5 and the HR ≤0.5 (Supplementary Figure S1). Nevertheless, most estimates of the adjusted HR were smaller than 1.0.

Discussion

In this large prospective cohort study, we have found nonsignificant inverse trends between total nut, tree nut, and peanut consumption and microscopically confirmed pancreatic cancer risk in males, but no or unclear associations in females. Increased peanut butter consumption was related to a significantly reduced MCPC risk in men, whereas this association was not clear in women. These associations were weaker in both genders when looking at total pancreatic cancer. Evidence for a nonlinear dose-response relation with MCPC was found for tree nut intake only. Furthermore, no significant interactions between total nut consumption and potentially effect modifying pancreatic cancer risk factors were identified.

The restriction to MCPC cases resulted in stronger associations between nut consumption and pancreatic cancer risk. This finding indicates that a lack of microscopic confirmation might have led to disease misclassification and to an attenuation of the results towards the null. Therefore, restriction to MCPC cases should be preferred.

Only two other studies have investigated the relation between nut consumption and pancreatic cancer, but neither looked at peanut butter consumption or at males specifically (16, 17). One case-control study found no association between 'nut and tasty snack' consumption and pancreatic cancer risk (16). However, because it did not examine nut consumption separately, its results cannot be directly compared to ours. In the prospective Nurses' Health Study (NHS), the HRs for a nut consumption frequency of ≥2x/week vs. never/almost never (95% CI) for pancreatic cancer in women were 0.68 (0.48-0.96), 0.89 (0.57-1.39), and 0.63 (0.39-1.03) for total nut, peanut, and other nut consumption, respectively (17). These results are in contrast with our results in women, for whom we found no or unclear associations. One possible explanation for this discrepancy might be the range of nut intake. In the women in our study, the mean total nut intake was 4.3 g/day, and the median intake in the highest consumption category 15.7 g/day. In the NHS, the number of

(28 g) servings per day in the highest consumption category was ≥0.20, which equals ≥5.6 g nuts/day (17). Therefore, it seems that the range of nut consumption is larger in the NLCS than in the NHS, and that the mean nut intake is higher. However, because the shape of the exposure-response curve for women in our study fluctuates over the entire intake range, the difference in nut intake between the two studies does not explain the discrepancy in the results. Furthermore, we included 161 female MCPC cases, whereas 424 (91% of the 466) female cases in the NHS were microscopically confirmed, resulting in more statistical power to detect significant differences.

Restricted cubic splines showed evidence for nonlinearity for tree nut intake in the NLCS, with a decreasing pancreatic cancer risk with increasing nut intake up to approximately 7.5 g tree nuts/day. We found no other studies that have examined the optimal daily dosage of nut consumption or the linearity of the relation between nut consumption and pancreatic cancer risk.

Differences in associations between men and women in our study might be explained by the mean amount of nuts consumed: on average, males consumed more total nuts than women, which was mainly due to their higher peanut intake. Tree nut and peanut butter intake were low in both sexes.

Although peanuts are botanically legumes, their nutrient composition is similar to that of tree nuts (12, 36). However, peanuts contain more proteins than, for example, almonds, cashew nuts, hazelnuts, and walnuts (36). Furthermore, compared with peanuts, peanut butter sold in the Netherlands in 1986 contained more vitamin B6, sodium, and partially hydrogenated fatty acids, but less niacin (36). Moreover, unlike nut consumers, respondents who consumed more peanut butter consumed less alcohol in our study.

In this study, we found a significant inverse trend in participants with a normal BMI, and no association in overweight participants. Nevertheless, the test for interaction by BMI was not significant ($P_{\text{Interaction}} = 0.082$). It has often been mentioned that nut consumption might contribute to weight gain and obesity because of their high caloric density, and that these negative health effects may outweigh the beneficial effects of nuts. However, we found no studies demonstrating a positive association between nut intake and weight gain or obesity. Recent prospective studies have actually shown that higher nut consumption is associated with less weight gain and a lower risk of obesity (37-39), which was seen in several cross-sectional studies and RCTs as well (39). Moreover, we also found that participants with a higher nut intake were leaner in this study (Table 1).

Even though our results have been adjusted for confounders, residual confounding by unmeasured confounders still might occur. Nevertheless, additional adjustments for the investigated potential confounders and the aMed score did not alter our results. Furthermore, the array-approach sensitivity analysis showed that most estimates of the adjusted HR were smaller than 1.0. Therefore, and because of the large number of potential confounders we have considered, it seems not very likely that an unmeasured confounder

exists that would dramatically alter our results.

The chance of reversed causation was minimized by excluding cases with prevalent cancer. Moreover, exclusion of pancreatic cancer cases diagnosed during the first two years of follow-up did not alter our results, and nut consumption of newly diagnosed cases was relatively constant over time. Furthermore, restricting the analyses of peanut butter to participants who had stated having had a constant peanut butter intake during the five years before baseline also showed that reversed causation is unlikely.

Strengths of this study include the prospective design, the high completeness of data, and the long duration of the follow-up, which make information and selection bias unlikely. Moreover, this was the first prospective cohort study that investigated the association of nut consumption and pancreatic cancer in men separately. Another strength is the high mean nut intake in our study population compared to other populations in Europe and Asia (40, 41). Potential limitations include possible measurement error, which might have attenuated the associations, and the fact that only baseline measurements were performed. However, nut intake appeared to be constant in other studies with repeated measurements (42), and a reproducibility study showed that dietary habits in our cohort were stable for at least five years (43). Furthermore, the complete case analysis approach may have resulted in biases if the missing data were not missing completely at random. For future research, we recommend to investigate the relation between nut consumption and pancreatic risk in participants without peanut or other nut allergies, because these allergies might potentially confound the observed associations. Unfortunately, we do not have information on these allergies.

In conclusion, total nut, tree nut, and peanut intake were associated with a nonsignificantly reduced MCPC risk in men, whereas peanut butter intake was associated with a significantly lowered MCPC risk. In women, no associations were found for total nut and tree nut consumption with MCPC, and unclear associations for peanut and peanut butter intake.

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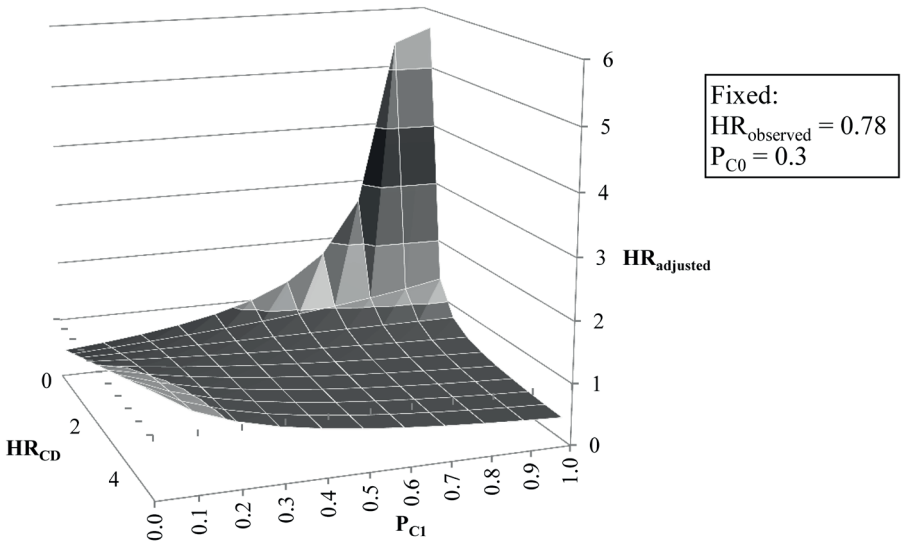

Supplementary Figure S1. Array approach sensitivity analysis to investigate the effect of an unmeasured confounder on the observed HR for microscopically confirmed pancreatic cancer for 5+ g total nut consumption/day vs. nonconsumers. $HR_{observed}$, observed association between total nut consumption and pancreatic cancer risk; $HR_{adjusted}$, fully adjusted HR for pancreatic cancer according to total nut consumption; HR_{CD}, association between the unmeasured confounder and pancreatic cancer risk; P_{C0}, prevalence of the confounder in the category of nonconsumers; P_{C1}, prevalence of the unmeasured confounder in the category of 5+ g total nut consumption/day.

Chapter 5

Nut and peanut butter consumption and the risk of lung cancer and its subtypes: A prospective cohort study

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Abstract

Objectives: Nut consumption has been associated with reduced cancer-related mortality, but evidence for a relation between nut intake and lung cancer risk is limited. We investigated the association between total nut, tree nut, peanut, and peanut butter intake and the risk of lung cancer and its subtypes in the Netherlands Cohort Study.

Materials and Methods: In 1986, dietary and lifestyle habits of 120,852 participants, aged 55-69 years, were measured with a questionnaire. After 20.3 years of follow-up, 3720 subcohort members and 2861 lung cancer cases were included in multivariable case-cohort analyses.

Results: Total nut intake was not significantly associated with total lung cancer risk in men or women. For small cell carcinoma, a significant inverse association with total nut intake was observed in men after controlling for detailed smoking habits (HR (95%CI) for 10+ g/day vs. nonconsumers: 0.62 (0.43-0.89), p-trend: 0.024). Inverse relations with small cell carcinoma were also found for tree nut and peanut intake in men in continuous analyses (HR (95%CI) per 5 g/day increment: 0.70 (0.53-0.93) and 0.93 (0.88-0.98), respectively). For the other lung cancer subtypes, no significant associations were seen in men. Nut intake was not related to the risk of lung cancer subtypes in women, and no associations were found for peanut butter in both sexes.

Conclusion: Increased nut intake might contribute to the prevention of small cell carcinoma in men. No significant associations were found in men for the other subtypes or total lung cancer, in women, or for peanut butter intake.

Introduction

In 2012, 1.8 million people were diagnosed with lung cancer worldwide, which accounted for 13% of all cancer diagnoses [1]. Survival rates of lung cancer are still poor, despite advances in its detection and treatment: the 5-year survival rate in the USA was 18% for total lung cancer and 4% for advanced lung cancer [2]. Unfortunately, minimally 50% of the patients are diagnosed when at an advanced disease stage [2].

The primary causative factor of lung cancer is tobacco smoking. Other factors, like age, sex, ethnicity, lung diseases, environmental and occupational exposures, and genetic factors may also influence lung cancer risk, as well as diet [3, 4]. Nuts have recently been hypothesized to conduct cancer-chemopreventive activities because of their antioxidant and anti-inflammatory effects [5]. Several studies have demonstrated inverse associations of nut intake with cancer-related mortality [6-9]. Nevertheless, evidence regarding the relation between nut consumption and lung cancer risk is limited to three cohort [10-12] and three case-control studies [12-14], and is inconclusive.

In two cohort studies, nut consumption was not significantly associated with lung cancer risk [10, 11]. A third cohort study, the NIH-AARP, observed inverse relations between nut consumption frequency and lung cancer risk across three major histologic subtypes [12]. In an accompanying Italian case-control study, nut consumption frequency was also significantly associated with a decreased lung cancer risk [12]. Two other case-control studies found no effects of nut consumption frequency, peanut consumption, or peanut butter consumption on lung cancer risk [13, 14].

Recently, we observed nonsignificant inverse associations between adherence to the Mediterranean diet and lung cancer risk [15]. Nut consumption appeared to contribute to this protective effect, especially in men, but was not studied thoroughly. Therefore, in this paper, we investigated in detail the relation between total nut, tree nut, peanut, and peanut butter consumption and the risk of lung cancer in men and women in the Netherlands Cohort Study (NLCS). Moreover, we examined whether this relation differs across the four major histologic lung cancer subtypes (adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and large cell carcinoma) and across never, former, and current smokers.

Materials and methods

Study design and cancer follow-up

The NLCS is a Dutch prospective population-based cohort study. At baseline (September 1986), 58,279 men and 62,573 women, aged 55-69 years, were included [16]. Participants agreed to participate by completing and returning a mailed, self-administered questionnaire on diet and other cancer risk factors. To improve the efficiency of the follow-up and data processing, a case-cohort method was applied by drawing a random subcohort (n = 5000) from the total cohort directly after baseline. Person-years at risk were calculated in the

subcohort, whereas cases originated from the entire cohort. The NLCS was approved by the institutional review boards of the Maastricht University and the Netherlands Organization for Applied Scientific Research.

Vital status information of the subcohort members was obtained biennially, and was 100% complete after 20.3 years of follow-up. Incident cancer cases were detected through record linkage with the Netherlands Cancer Registry and the Dutch Pathology Registry (PALGA), with a completeness >95% after the follow-up period [17, 18].

In the current analyses, 3720 subcohort members and 2861 incident lung cancer cases (ICD-O-3 code C34) diagnosed between September 1986 and December 2006 were included after applying the exclusion criteria: participants with prevalent cancer (except skin cancer), with inconsistent or incomplete dietary data, or with missing data on confounding variables were excluded, as were cases with non-carcinoma, in situ lung cancers, or lung cancers without microscopic confirmation (Supplementary Figure S1).

Exposure assessment

The 11-page self-administered baseline questionnaire measured dietary factors and other cancer risk factors, e.g. detailed smoking habits, physical activity, and anthropometrics. Habitual diet in the year preceding baseline was assessed using a validated 150-item semi-quantitative food frequency questionnaire (FFQ) [19]. Participants filled in how often they consumed 'peanuts', 'other, mixed nuts' (tree nuts), and 'peanut butter', which could range from 'never or less than 1x/month' to '6-7x/week'. Participants also filled in the number of standard portions sizes they consumed per intake. The assumed standard portion sizes were 28 grams for tree nuts and peanuts, and 15 grams per slice of bread for peanut butter. Mean daily intakes were calculated by multiplying intake frequencies and portion sizes. Total nut intake was the sum of tree nut and peanut intake.

Statistical analysis

To evaluate the relation between nut consumption and the risk of total lung cancer, adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and large cell carcinoma, we estimated hazard ratios (HRs) and 95% confidence intervals (95%CIs) using age-adjusted and multivariable-adjusted Cox proportional hazards models. Person-years in the subcohort were calculated from baseline until cancer diagnosis, death, emigration, loss to follow-up, or end of follow-up. Standard errors were calculated using the robust Huber-White sandwich estimator to account for the additional variance introduced by sampling from the entire cohort [20]. Schoenfeld residuals, log-log survival plots, and time-varying covariates were used to test the proportional hazard assumption, which was met for the exposure variables. If the assumption was violated for confounders, time-varying covariates were included in the model.

The associations between nut and peanut butter consumption and lung cancer risk were analyzed on a categorical and continuous scale, for men and women separately. Total nut consumption was categorized into 0, 0.1-<5, 5-<10, and 10+ g/day, peanut and peanut

butter consumption into 0, 0.1-<5, and 5+ g/day, and tree nut intake into 0 and 0.1+ g/day, because of the limited number of cases in the higher intake categories. Intake of 0 g/day was the reference category. Sex-specific median values per intake category in the subcohort were fitted as continuous variables in regression models to perform trend tests. Continuous analyses were performed per 5 g/day increment.

We ran three models per nut exposure to correct for predefined confounders, which were literature-based and included in the models independent of their effect on the estimates: in the age-adjusted model, we adjusted for age at baseline (years; continuous). In the smoking-adjusted model, we additionally adjusted for cigarette smoking status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered). In the fully adjusted model, we additionally adjusted for body mass index (BMI; <18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (primary or lower vocational (low), secondary or medium vocational (medium), higher vocational or university (high)), family history of lung cancer (yes, no), history of chronic bronchitis (yes, no), daily energy intake (kcal/day; continuous), alcohol consumption (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), and alternate Mediterranean (aMED) diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points). Other considered potential confounders were height, nutritional supplement use, and history of tuberculosis and asthma. Because these variables did not change the HRs with ≥10% when using a backward stepwise selection procedure, they were not included in the final model.

Restricted cubic spline analyses with three fixed knots at 0, 5, and 10 g nut intake/day were performed to evaluate the linearity of the exposure-response relation between nut intake and lung cancer risk. Heterogeneity in associations with nut intake across the four histologic lung cancer subtypes was tested using a competing risk procedure [21], which estimates standard errors using a bootstrapping method designed for the case-cohort approach [22].

To investigate interactions by cancer risk factors and potential residual confounding, we stratified the associations by cigarette smoking status (never, former, current) and frequency (1-<20 or 20+ cigarettes/day). In an additional analysis, we stratified the results by BMI (18.5-<25, ≥25 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), alcohol consumption (0, 0.1-15, ≥15 g/day), educational level (low, medium, high), aMED score excluding alcohol and nuts (0-2, 3-4, 5-7 points), and family history of lung cancer (yes, no). Participants with a BMI <18.5 kg/m^2 were excluded from the latter analysis, because of their limited numbers. Interactions were tested by including cross-product terms in the models and performing Wald tests.

In sensitivity analyses, we excluded the first two years of follow-up to investigate potential reversed causation, and we restricted the analysis of peanut butter to those with a constant peanut butter intake in the five years before baseline. To investigate potential residual confounding, we adjusted the associations for intake of fruits, vegetables, milk, milk products, cheese, and red and processed meat instead of for the aMED score excluding alcohol and nuts. Moreover, tree nut, peanut, and peanut butter intakes were mutually

adjusted in additional analyses.

All analyses were performed in Stata 14 software (StataCorp. 2015. College Station, TX). P-values were tested two-sided, with values <0.05 considered statistically significant.

Results

Squamous cell carcinoma was the most commonly diagnosed lung cancer subtype in men (38.7%), followed by adenocarcinoma (22.2%). In women, the most commonly diagnosed subtype was adenocarcinoma (33.5%), followed by squamous cell carcinoma (23.2%) (Table 1). Histology was unspecified in 7.2% and 9.4% of the male and female cases, respectively.

The mean (SD) total nut intake was 7.1 (13.1) g/day in male lung cancer cases, which was somewhat lower than in the subcohort (7.9 (13.7) g/day) (Table 1). In women, the mean (SD) total nut intake in cases was 4.9 (10.8) g/day, which was slightly higher than in the subcohort (4.4 (8.5) g/day). In men, the median (IQR) total nut intake was 2.0 (0.0-8.5) in lung cancer cases and 2.8 (0.0-9.0) in subcohort members. In women, these values were 1.0 (0.0-4.9) and 1.6 (0.0-4.9), respectively. Tree nut, peanut, and peanut butter consumption were, on average, lower in male cases than in the subcohort. In female cases, tree nut and peanut butter intake were somewhat lower than in the subcohort, whereas peanut intake was higher.

Regarding other baseline characteristics (Table 1), male cases were, on average, older than subcohort members, whereas female cases were younger. Only 13.6% of the male subcohort members had never smoked. Cases were more often former or current smokers, more often reported a positive family history of lung cancer, consumed more alcohol, and had a lower aMED score (excluding alcohol and nuts) than subcohort members. In addition, cases, except female small cell carcinoma cases, more often reported a history of chronic bronchitis and had a higher average daily energy intake than the subcohort. Furthermore, cases, except female adenocarcinoma cases, were more often lower educated than subcohort members.

Age-adjusted and multivariable-adjusted associations between nut consumption and total lung cancer risk are presented in Table 2. In the age-adjusted analyses, statistically significant inverse associations with total lung cancer risk were found for total nuts, tree nuts, and peanuts in men, and non-statistically significant inverse associations in women. For peanut butter intake, nonsignificant inverse associations were observed in both sexes. When additionally adjusting for cigarette smoking status, frequency, and duration, most associations became weaker. After full adjustment, the associations attenuated even more, and some became positive in women.

Total nut intake was not significantly associated with total lung cancer risk in men and women (HR (95%CI) for 10+ g/day vs. nonconsumers: 0.83 (0.67-1.04), p-trend: 0.184, and 0.91 (0.58-1.43), p-trend: 0.720, respectively). For tree nut and peanut intake, nonsignificant

Table 1. Baseline characteristics (mean (SD) or %) of subcohort members and lung cancer cases in the Netherlands Cohort Study, 1986-2006

	Subcohort	Total lung cancer	Adenocarcinoma	Squamous cell carcinoma	Small cell carcinoma	Large cell carcinoma
Men						
N	1,834	2,413	535	933	395	376
Age (years)	61.2 (4.2)	61.7 (4.2)	61.4 (3.9)	61.8 (4.3)	61.7 (4.1)	61.9 (4.2)
Body mass index (kg/m^2)	24.9 (2.6)	24.9 (2.7)	24.7 (2.6)	25.0 (2.6)	25.1 (2.6)	24.8 (2.8)
Never cigarette smoker (%)	13.6	3.9	3.0	4.5	3.3	4.8
University or higher vocational education (%)	20.3	13.8	18.1	12.2	12.2	11.2
Nonoccupational physical activity (min/day)	81.0 (67.4)	78.9 (68.4)	76.0 (63.3)	77.7 (68.1)	81.9 (76.2)	80.6 (67.6)
Family history of lung cancer (%)	9.4	12.9	9.9	14.8	13.4	12.8
History of chronic bronchitis (%)	7.3	10.3	9.0	12.1	9.1	8.2
Daily energy intake (kcal)	2,167 (499)	2,185 (500)	2,192 (519)	2,181 (501)	2,175 (486)	2,191 (492)
Total nut intake (g/day)	7.9 (13.7)	7.1 (13.1)	7.7 (14.4)	7.6 (13.4)	5.4 (9.4)	6.3 (9.7)
Tree nut intake (g/day)	1.0 (3.4)	0.8 (2.8)	0.8 (2.5)	0.9 (3.6)	0.5 (1.3)	0.7 (2.3)
Peanut intake (g/day)	6.9 (13.0)	6.4 (12.4)	6.9 (13.7)	6.7 (12.4)	4.9 (9.0)	5.6 (9.4)
Peanut butter intake (g/day)	1.4 (4.2)	1.2 (4.0)	1.5 (4.6)	1.3 (4.3)	1.0 (2.7)	1.3 (3.4)
Alcohol intake (g/day)	15.1 (17.1)	19.2 (19.7)	19.0 (19.4)	19.7 (20.2)	19.0 (19.0)	18.6 (19.8)
aMED score (excl. alcohol and nuts) of 5-7 pts (%)	22.6	18.1	18.9	18.0	14.4	20.7
Women						
N	1,886	448	150	104	83	69
Age (years)	61.4 (4.2)	60.7 (4.0)	61.0 (4.2)	60.1 (3.9)	60.1 (3.9)	61.2 (4.1)
Body mass index (kg/m^2)	25.0 (3.5)	24.3 (3.4)	24.3 (3.1)	24.4 (3.6)	25.0 (3.6)	23.3 (3.4)
Never cigarette smoker (%)	58.9	23.0	41.3	12.5	6.0	15.9
University or higher vocational education (%)	9.5	9.2	12.7	6.7	7.2	7.3
Nonoccupational physical activity (min/day)	65.5 (50.6)	64.3 (59.1)	57.3 (50.8)	75.7 (68.4)	72.4 (72.3)	63.3 (53.0)
Family history of lung cancer (%)	10.5	13.6	13.3	15.4	14.5	11.6
History of chronic bronchitis (%)	5.1	8.9	8.7	17.3	2.4	7.3
Daily energy intake (kcal)	1,684 (389)	1,700 (385)	1,724 (383)	1,716 (379)	1,642 (399)	1,706 (396)
Total nut intake (g/day)	4.4 (8.5)	4.9 (10.8)	4.8 (10.9)	4.6 (8.5)	3.7 (7.5)	6.9 (16.3)
Tree nut intake (g/day)	1.1 (4.0)	0.9 (2.9)	1.2 (3.8)	1.0 (2.9)	0.5 (1.6)	0.9 (2.4)
Peanut intake (g/day)	3.3 (7.0)	4.0 (9.5)	3.6 (8.1)	3.6 (7.7)	3.2 (7.1)	6.0 (15.8)
Peanut butter intake (g/day)	1.2 (3.6)	1.0 (2.9)	0.8 (2.5)	1.2 (3.1)	1.2 (3.8)	1.3 (2.9)
Alcohol intake (g/day)	6.0 (9.5)	9.4 (14.4)	9.4 (13.5)	10.9 (16.9)	8.5 (13.6)	8.6 (13.7)
aMED score (excl. alcohol and nuts) of 5-7 pts (%)	25.8	17.9	20.7	17.3	16.9	15.9

inverse associations were found in men, and no relations in women. Peanut butter intake was not associated with total lung cancer risk in both sexes. Although we found no significant interactions between the nut variables and sex (p-interaction ≥0.403), we chose to present the results for men and women separately because of the substantial differences in estimates between the sexes.

In restricted cubic spline analyses with fixed knots at 0, 5, and 10 g nut intake/day, the exposure-response relations with total lung cancer risk were linear for all nut variables in both sexes (Figure 1). Based on the Akaike Information Criterion (AIC) score, the model fit did not improve when using additional knots or different knot positions.

Across the four histologic lung cancer subtypes, the multivariable-adjusted associations with nut intake varied slightly in strength (Table 3), but the heterogeneity tests were not significant in men and women (p-heterogeneity = 0.090 and 0.998, respectively). For small cell carcinoma, a significant inverse association with total nut intake was observed in men (HR (95%CI) for 10+ g/day vs nonconsumers: 0.62 (0.43-0.89), p-trend: 0.024). For adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, nonsignificant inverse trends with total nut intake were seen in men. In continuous analyses, a (borderline) significant association with large cell carcinoma was found (HR (95%CI) per 5 g/day increment: 0.95 (0.90-1.00)). In women, no or nonsignificant positive associations with total nut intake were found for the four subtypes. For tree nut intake, nonsignificant inverse associations with all lung cancer subtypes were observed in men. In continuous analyses, the HR (95%CI) per 5 g tree nuts/day increment was 0.70 (0.53-0.93) for small cell carcinoma. No significant associations were found for tree nut intake for the four subtypes in women. For peanut intake, nonsignificant inverse associations with all lung cancer subtypes were seen in men, and a significant inverse association for small cell carcinoma in continuous analyses (HR (95%CI) per 5 g/day increment: 0.93 (0.88-0.98)). In women, no or nonsignificant positive associations were found for all subtypes for peanut intake. Peanut butter intake was not significantly associated with the lung cancer subtypes in both sexes.

Stratification of the relation between total nut intake and total lung cancer by smoking status in men showed nonsignificant inverse associations in never smokers, significant inverse associations in former smokers, and no relation in current smokers (p-trend: 0.854, 0.007, and 0.843, respectively) (Table 4). To increase statistical power, the two highest intake categories were merged into one category of 5+ g/day for these stratified analyses. The test for interaction by smoking status was significant in men (p-interaction: 0.042). In women, the relation between total nut intake and total lung cancer was unclear in never smokers, non-significantly inverse in former smokers, and non-significantly positive in current smokers (p-trend: 0.452, 0.549, and 0.937, respectively). The test for interaction was not significant (p-interaction: 0.387). After further stratification by smoking frequency, the HRs in male former smokers were stronger inverse in lighter smokers (1-<20 cigarettes/day) than in heavy smokers (20+ cigarettes/day) (p-interaction: 0.005). In male current smokers, there was no significant interaction by smoking frequency (p-interaction: 0.111), although stronger inverse associations were seen in lighter smokers. In female former and current

Table 2. Age- and sex-adjusted and multivariable-adjusted HRs (and 95%CIs) for total lung cancer according to nut consumption; NLCS, 1986-2006

	Median intake[a]		Men					Women				
	Men	Women	Person-years	Cases	Age-adjusted HR (95%CI)	Multivariable-adjusted HR[b] (95%CI)	Multivariable-adjusted HR[c] (95%CI)	Person-years	Cases	Age-adjusted HR (95%CI)	Multivariable-adjusted HR[b] (95%CI)	Multivariable-adjusted HR[c] (95%CI)
Total nuts (g/day)												
0	0.0	0.0	8,696	897	1.00 (reference)	1.00 (reference)	1.00 (reference)	13,308	196	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	2.5	2.1	9,613	733	0.76 (0.65-0.88)	0.89 (0.74-1.07)	0.87 (0.72-1.06)	12,257	150	0.81 (0.63-1.02)	1.04 (0.77-1.39)	1.10 (0.81-1.49)
5-<10	8.5	7.8	3,931	285	0.73 (0.60-0.90)	0.89 (0.70-1.15)	0.91 (0.70-1.19)	3,794	49	0.82 (0.58-1.18)	1.01 (0.66-1.57)	1.17 (0.73-1.87)
10+	21.4	15.7	6,965	498	0.73 (0.62-0.87)	0.80 (0.66-0.98)	0.83 (0.67-1.04)	4,280	53	0.80 (0.57-1.13)	0.85 (0.56-1.29)	0.91 (0.58-1.43)
P_{trend}					0.007	0.058	0.184			0.273	0.455	0.720
Continuous, per 5 g/day increment					0.98 (0.95-1.00)	0.97 (0.95-1.00)	0.98 (0.95-1.01)			1.02 (0.96-1.08)	1.02 (0.95-1.09)	1.02 (0.95-1.10)
Tree nuts (g/day)												
0	0.0	0.0	21,040	1,892	1.00 (reference)	1.00 (reference)	1.00 (reference)	23,457	332	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1+	1.6	2.1	8,165	521	0.72 (0.62-0.83)	0.83 (0.70-0.99)	0.87 (0.72-1.04)	10,182	116	0.79 (0.62-1.00)	0.95 (0.71-1.26)	1.01 (0.74-1.37)
Continuous, per 5 g/day increment					0.83 (0.73-0.95)	0.89 (0.77-1.03)	0.90 (0.78-1.05)			0.90 (0.74-1.10)	0.93 (0.74-1.17)	0.92 (0.72-1.17)
Peanuts (g/day)												
0	0.0	0.0	9,802	963	1.00 (reference)	1.00 (reference)	1.00 (reference)	15,693	217	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	2.5	2.1	10,477	786	0.80 (0.68-0.92)	0.89 (0.74-1.06)	0.88 (0.73-1.06)	12,519	156	0.87 (0.69-1.10)	1.06 (0.80-1.40)	1.15 (0.86-1.55)
5+	12.8	10.7	8,926	664	0.81 (0.69-0.95)	0.85 (0.70-1.02)	0.89 (0.72-1.08)	5,427	75	0.95 (0.71-1.28)	0.96 (0.67-1.39)	1.05 (0.70-1.56)
P_{trend}					0.051	0.119	0.371			0.833	0.808	0.886
Continuous, per 5 g/day increment					0.99 (0.96-1.01)	0.98 (0.95-1.00)	0.98 (0.95-1.01)			1.05 (0.97-1.12)	1.03 (0.96-1.11)	1.04 (0.96-1.13)
Peanut butter (g/day)												
0	0.0	0.0	20,916	1,809	1.00 (reference)	1.00 (reference)	1.00 (reference)	24,516	334	1.00 (reference)	1.00 (reference)	1.00 (reference)
0.1-<5	1.2	1.2	5,067	366	0.87 (0.73-1.03)	0.91 (0.75-1.11)	0.98 (0.79-1.20)	5,927	79	0.96 (0.73-1.26)	0.99 (0.71-1.38)	1.03 (0.72-1.45)
5+	9.6	6.9	3,223	238	0.89 (0.72-1.09)	0.91 (0.71-1.17)	0.92 (0.70-1.19)	3,197	35	0.78 (0.53-1.15)	0.91 (0.58-1.41)	1.03 (0.65-1.62)
P_{trend}					0.258	0.470	0.519			0.205	0.668	0.906
Continuous, per 5 g/day increment					0.95 (0.87-1.03)	0.98 (0.88-1.08)	0.98 (0.88-1.10)			0.89 (0.75-1.06)	0.91 (0.76-1.09)	0.95 (0.79-1.13)

[a] Median intake in the subcohort

[b] Adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered))

[c] Adjusted for [b] and body mass index (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of lung cancer (yes, no), history of chronic bronchitis (yes, no), daily energy intake (kcal/day; continuous), alcohol consumption (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), and alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points)

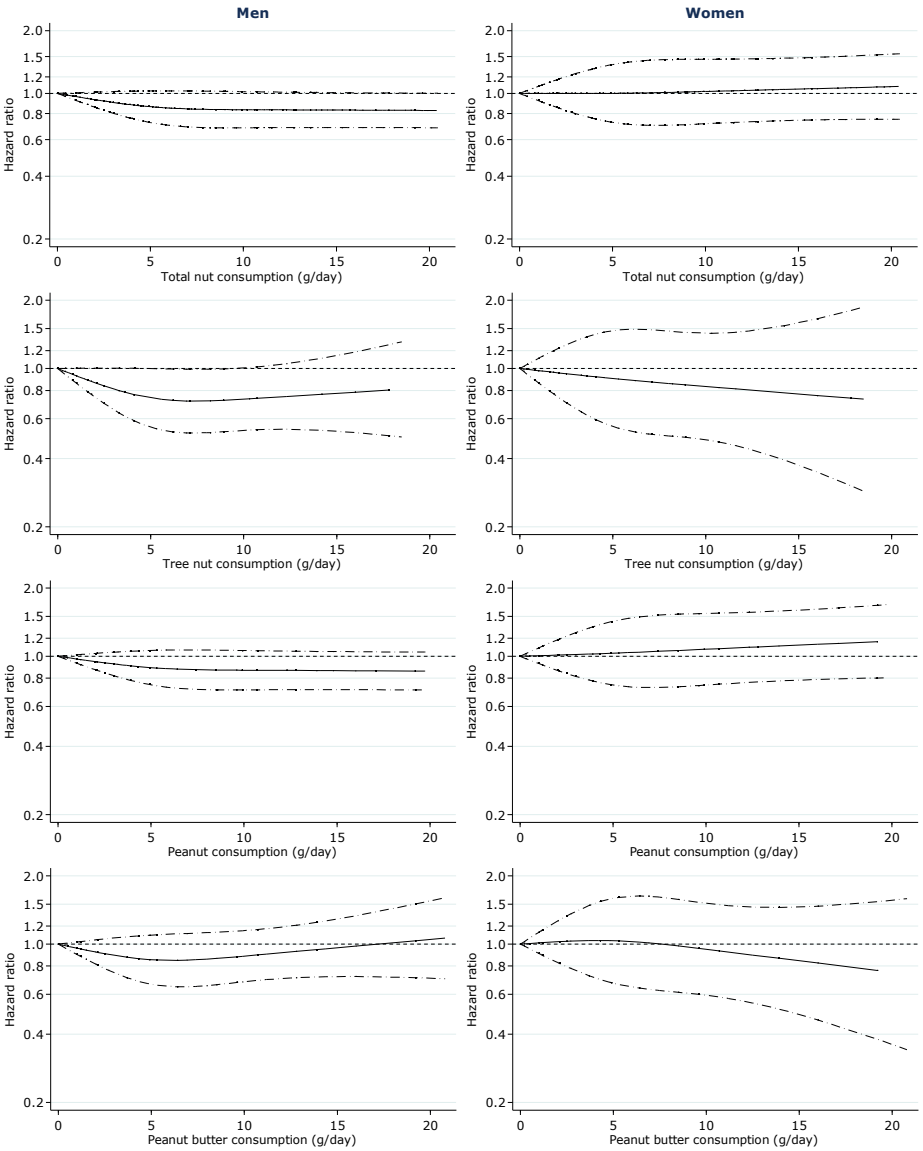

Figure 1. Nonparametric regression curves for the associations between total lung cancer risk and intake of total nuts, tree nuts, peanuts, and peanut butter in men and women separately. Solid lines represent multivariable-adjusted HRs; dashed lines represent 95%CIs. HRs were calculated using restricted cubic spline models with three fixed knots at intakes of 0, 5, and 10 g/day, and were adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered) and duration (years; continuous, centered)), body mass index (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of lung cancer (yes, no), history of chronic bronchitis (yes, no), daily energy intake (kcal/day; continuous), alcohol consumption (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), and alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points). P-values for nonlinearity for total nut, tree nut, peanut, and peanut butter intake were 0.143, 0.110, 0.257, and 0.185 in men, and 0.888, 0.946, 0.959, and 0.625 in women, respectively.

smokers, no interactions by smoking frequency were found.

Interactions between nut intake and smoking characteristics were also investigated for the lung cancer subtypes. Because of the low number of never smoking male cases, interactions by smoking habits for the subtypes were tested on a continuous scale. (Borderline) significant inverse associations of total nut intake with small cell carcinoma were observed in male former and current smokers, and a stronger, but nonsignificant inverse association in male never smokers (p-interaction: 0.574) (Table 5). When additionally stratifying by smoking frequency, no association was observed in male former lighter smokers, and a significant inverse association in male former heavy smokers (p-interaction: 0.075). In male current smokers, nonsignificant inverse associations were found in both strata of smoking frequency (p-interaction: 0.332). For small cell carcinoma, also no significant interactions between smoking frequency and tree nut or peanut intake were observed in male former and current smokers (data not shown). The associations with small cell carcinoma risk in male former heavy smokers and male lighter and heavy current smokers were stronger inverse for tree nut intake than for total nut intake (data not shown). For peanut intake, comparable estimates as for total nut intake were found when stratifying by smoking frequency in male current and former smokers (data not shown). No significant interactions by smoking characteristics were found for small cell carcinoma in women or for the other subtypes in both sexes, although the number of female cases in each stratum of smoking frequency was very small (data not shown).

For the other considered lung cancer risk factors we observed no significant interactions in both sexes (Supplementary Table S1). In sensitivity analyses, adjustment for fruit, vegetable, milk, milk product, cheese, and red and processed meat intake resulted in similar associations as when adjusting for the aMED score excluding alcohol and nuts (data not shown). Mutual adjustment for tree nut, peanut, and peanut butter intake also did not change the estimates (data not shown). Excluding the first two years of follow-up did not importantly alter the estimates, and neither did restricting the analyses to participants with a constant peanut butter intake in the five years before baseline (data not shown).

Discussion

In this large prospective cohort study, increased total nut, tree nut, and peanut intake was associated with a non-significantly decreased lung cancer risk in men. The inverse relation for total nut intake in men differed significantly across strata of smoking characteristics, and was strongest in never and former lighter smokers (1-<20 cigarettes/day). The risk of small cell carcinoma was significantly reduced in men with increasing nut intake, after controlling for smoking status, frequency, and duration. Significant inverse relations with small cell carcinoma in men were also seen for tree nut and peanut intake in continuous analyses. For adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, nonsignificant inverse associations were found for all nut exposures in men. In women, nut intake was not related to the risk of lung cancer, nor its subtypes. Peanut butter was also not associated with lung

Table 3. Multivariable-adjusted HRs (and 95%CIs) for the four major histologic lung cancer subtypes according to nut consumption; NLCS, 1986-2006

			Adenocarcinoma		Squamous cell carcinoma		Small cell carcinoma		Large cell carcinoma	
	Median intake[a]	Person-years	N cases	Multivariable-adjusted HR[b] (95%CI)	N cases	Multivariable-adjusted HR[b] (95%CI)	N cases	Multivariable-adjusted HR[b] (95%CI)	N cases	Multivariable-adjusted HR[b] (95%CI)
Men										
Total nuts (g/day)										
0	0.0	8,696	195	1.00 (reference)	347	1.00 (reference)	162	1.00 (reference)	137	1.00 (reference)
0.1-<5	2.5	9,613	169	0.90 (0.69-1.18)	274	0.85 (0.67-1.08)	116	0.82 (0.60-1.12)	118	0.89 (0.65-1.21)
5-<10	8.5	3,931	52	0.71 (0.48-1.05)	109	0.91 (0.66-1.26)	56	1.12 (0.75-1.67)	44	0.91 (0.59-1.38)
10+	21.4	6,965	119	0.84 (0.62-1.14)	203	0.91 (0.70-1.19)	61	0.62 (0.43-0.89)	77	0.82 (0.57-1.18)
P_{trend}				0.317		0.783		0.024		0.373
Continuous, per 5 g/day increment				0.99 (0.95-1.03)		1.00 (0.96-1.03)		0.92 (0.87-0.98)		0.95 (0.90-1.00)
Tree nuts (g/day)										
0	0.0	21,040	417	1.00 (reference)	729	1.00 (reference)	308	1.00 (reference)	300	1.00 (reference)
0.1+	1.6	8,165	118	0.84 (0.65-1.09)	204	0.89 (0.71-1.12)	87	0.98 (0.72-1.32)	76	0.79 (0.58-1.07)
Continuous, per 5 g/day increment				0.89 (0.72-1.10)		1.01 (0.84-1.21)		0.70 (0.53-0.93)		0.83 (0.63-1.09)
Peanuts (g/day)										
0	0.0	9,802	206	1.00 (reference)	373	1.00 (reference)	171	1.00 (reference)	153	1.00 (reference)
0.1-<5	2.5	10,477	181	0.93 (0.71-1.20)	295	0.86 (0.68-1.08)	129	0.87 (0.65-1.18)	120	0.82 (0.61-1.10)
5+	12.8	8,926	148	0.86 (0.65-1.15)	265	0.94 (0.74-1.21)	95	0.77 (0.55-1.07)	103	0.85 (0.61-1.18)
P_{trend}				0.357		0.928		0.144		0.503
Continuous, per 5 g/day increment				0.99 (0.95-1.04)		1.00 (0.96-1.03)		0.93 (0.88-0.98)		0.96 (0.91-1.01)
Peanut butter (g/day)										
0	0.0	20,916	381	1.00 (reference)	712	1.00 (reference)	305	1.00 (reference)	275	1.00 (reference)
0.1-<5	1.2	5,067	93	1.10 (0.82-1.46)	134	0.94 (0.72-1.23)	50	0.85 (0.60-1.22)	64	1.13 (0.81-1.60)
5+	9.6	3,223	61	1.14 (0.80-1.62)	87	0.85 (0.61-1.18)	40	0.97 (0.64-1.48)	37	0.91 (0.59-1.41)
P_{trend}				0.488		0.336		0.903		0.675
Continuous, per 5 g/day increment				1.07 (0.93-1.23)		0.99 (0.85-1.15)		0.89 (0.73-1.08)		0.98 (0.82-1.17)
Women										
Total nuts (g/day)										
0	0.0	13,308	65	1.00 (reference)	43	1.00 (reference)	40	1.00 (reference)	28	1.00 (reference)
0.1-<5	2.1	12,257	51	0.98 (0.64-1.49)	36	1.31 (0.73-2.37)	28	1.15 (0.64-2.06)	22	1.26 (0.67-2.37)

(Continued)			Adenocarcinoma		Squamous cell carcinoma		Small cell carcinoma		Large cell carcinoma	
	Median intake[a]	Person-years	N cases	Multivariable-adjusted HR[b] (95%CI)	N cases	Multivariable-adjusted HR[b] (95%CI)	N cases	Multivariable-adjusted HR[b] (95%CI)	N cases	Multivariable-adjusted HR[b] (95%CI)
5-<10	7.8	3,794	17	1.02 (0.54-1.90)	12	1.64 (0.68-3.97)	8	0.92 (0.33-2.56)	9	1.74 (0.68-4.46)
10+	15.7	4,280	17	0.72 (0.38-1.35)	13	1.13 (0.48-2.69)	7	0.73 (0.28-1.94)	10	1.28 (0.54-3.03)
P_{trend}				0.338		0.693		0.473		0.509
Continuous, per 5 g/day increment				0.99 (0.88-1.12)		1.02 (0.90-1.15)		0.98 (0.84-1.15)		1.10 (0.97-1.25)
Tree nuts (g/day)										
0	0.0	23,457	107	1.00 (reference)	76	1.00 (reference)	68	1.00 (reference)	48	1.00 (reference)
0.1+	2.1	10,182	43	1.01 (0.66-1.55)	28	1.13 (0.62-2.08)	15	0.70 (0.35-1.39)	21	1.40 (0.75-2.59)
Continuous, per 5 g/day increment				0.99 (0.78-1.27)		0.92 (0.61-1.39)		0.63 (0.30-1.36)		0.93 (0.63-1.38)
Peanuts (g/day)										
0	0.0	15,693	72	1.00 (reference)	46	1.00 (reference)	42	1.00 (reference)	35	1.00 (reference)
0.1-<5	2.1	12,519	54	1.07 (0.71-1.59)	41	1.67 (0.92-3.02)	29	1.18 (0.65-2.13)	20	1.01 (0.56-1.83)
5+	10.7	5,427	24	0.85 (0.49-1.48)	17	1.27 (0.57-2.82)	12	1.05 (0.47-2.34)	14	1.26 (0.58-2.74)
P_{trend}				0.526		0.664		0.943		0.545
Continuous, per 5 g/day increment				0.99 (0.87-1.13)		1.04 (0.92-1.18)		1.01 (0.87-1.18)		1.12 (0.98-1.28)
Peanut butter (g/day)										
0	0.0	24,516	117	1.00 (reference)	73	1.00 (reference)	63	1.00 (reference)	47	1.00 (reference)
0.1-<5	1.2	5,927	22	0.83 (0.50-1.38)	23	1.28 (0.70-2.35)	13	0.84 (0.39-1.80)	13	1.29 (0.63-2.68)
5+	6.9	3,197	11	0.79 (0.40-1.56)	8	1.17 (0.48-2.84)	7	1.00 (0.39-2.59)	9	1.92 (0.81-4.54)
P_{trend}				0.475		0.647		0.967		0.130
Continuous, per 5 g/day increment				0.83 (0.60-1.15)		1.02 (0.78-1.35)		0.97 (0.66-1.42)		1.06 (0.81-1.38)

[a] In the subcohort

[b] Adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), body mass index (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of lung cancer (yes, no), history of chronic bronchitis (yes, no), daily energy intake (kcal/day; continuous), alcohol consumption (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), and alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points)

cancer risk in both sexes.

In contrast to our study, no relation between nut intake and lung cancer risk was found in the Adventist Health Study, although no estimates were reported [10]. In the COSMOS lung cancer screening study, a nonsignificant inverse relation was observed among heavy smokers [11]. However, these cohort studies had small sample sizes and relatively short follow-up periods, and they did not stratify by sex or histologic subtype.

A third cohort study, the NIH-AARP, with 18,533 incident lung cancer cases found significant inverse associations between nut consumption frequency and risk of adenocarcinoma, squamous cell carcinoma, and small cell carcinoma in both sexes combined, after controlling for smoking characteristics [12]. They also observed that lighter smokers may benefit most from higher nut consumption, and sensitivity analyses suggested that nut intake might be most protective against small cell carcinoma. Furthermore, the authors stated that similar associations were found in men and women. These results largely correspond with our findings, although we observed substantial differences between the sexes, and nonsignificant inverse associations for adenocarcinoma and squamous cell carcinoma in men. The latter might be explained by the higher statistical power in the NIH-AARP.

In an accompanying Italian case-control study, nut consumption frequency was also significantly associated with a decreased lung cancer risk [12]. A Hawaiian case-control study found no association between peanut and peanut butter intake and lung cancer risk [14], and another Italian case-control study also observed no effect of nut consumption frequency on lung cancer risk [13].

In our study, the relation between nut intake and lung cancer risk differed substantially between the sexes. This might be explained by the lower mean nut intake in women (4.4 g/day) than in men (7.9 g/day). Only one other cohort [12] and two case-control studies [12, 14] performed sex-stratified analyses, and observed no differences between men and women. Because these studies did not report sex-specific mean nut intakes, it is difficult to compare them to our study. Other possible explanations for the observed differences between men and women might be residual confounding by smoking characteristics or hormonal mechanisms that might contribute to lung carcinogenesis [23, 24].Because there is no clear explanation for the observed dissimilarities, additional research investigating sex-differences in the relation between nut intake and lung cancer risk is required.

The inverse relation with nut intake in men was strongest for small cell carcinoma, after controlling for smoking habits. This finding is important, because small cell carcinoma is characterized by its rapid growth and early metastatic spread [25]. Moreover, it has the strongest relation with smoking of all subtypes [26-28].

For total lung cancer and small cell carcinoma, the inverse association with total nut intake in men was strongest in never and former smokers. Moreover, for total lung cancer, the inverse association was stronger in lighter smokers than in heavy smokers. Stronger inverse

Table 4. Total lung cancer risk according to total nut intake, in multivariable-adjusted analyses[a], in strata of smoking status and smoking frequency; the Netherlands Cohort Study, 1986-2006

	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
Total lung cancer					
Men					
Smoking status					
Never					
Cases/person-time at risk (years)	44/1,296	21/1,598	30/1,451		
HR (95%CI)	1 (ref)	0.34 (0.15-0.75)	0.63 (0.27-1.46)	0.854	0.042
Former					
Cases/person-time at risk (years)	291/4,433	256/5,194	269/6,164		
HR (95%CI)	1 (ref)	0.91 (0.68-1.20)	0.68 (0.51-0.91)	0.007	
Current					
Cases/person-time at risk (years)	562/2,967	456/2,822	484/3,280		
HR (95%CI)	1 (ref)	0.91 (0.68-1.20)	0.99 (0.75-1.31)	0.843	
Smoking frequency					
Former cigarette smokers					
1-<20 cigarettes/day					
Cases/person-time at risk (years)	119/2,350	91/3,225	106/3,071		
HR (95%CI)	1 (ref)	0.56 (0.36-0.85)	0.61 (0.39-0.96)	0.164	0.005
20+ cigarettes/day					
Cases/person-time at risk (years)	172/2,083	165/1,969	163/3,093		
HR (95%CI)	1 (ref)	1.22 (0.82-1.81)	0.71 (0.47-1.06)	0.021	
Current cigarette smokers					
1-<20 cigarettes/day					
Cases/person-time at risk (years)	259/1,638	213/1,841	222/1,839		
HR (95%CI)	1 (ref)	0.68 (0.47-0.99)	0.89 (0.61-1.32)	0.935	0.111
20+ cigarettes/day					
Cases/person-time at risk (years)	303/1,329	243/981	262/1,441		
HR (95%CI)	1 (ref)	1.16 (0.76-1.78)	0.94 (0.63-1.42)	0.585	
Women					
Smoking status					
Never					
Cases/person-time at risk (years)	47/8,430	30/7,502	26/4,226		
HR (95%CI)	1 (ref)	0.75 (0.46-1.23)	1.14 (0.66-1.96)	0.452	0.387
Former					
Cases/person-time at risk (years)	23/1,957	27/2,736	20/2,249		
HR (95%CI)	1 (ref)	0.88 (0.41-1.91)	0.75 (0.30-1.88)	0.549	
Current					
Cases/person-time at risk (years)	126/2,921	93/2,019	56/1,600		
HR (95%CI)	1 (ref)	1.39 (0.87-2.21)	1.03 (0.59-1.80)	0.937	
Smoking frequency					
Former cigarette smokers					
1-<20 cigarettes/day					
Cases/person-time at risk (years)	12/1,494	16/2,228	8/1,844		
HR (95%CI)	1 (ref)	1.98 (0.68-5.78)	0.81 (0.23-2.86)	0.350	0.676
20+ cigarettes/day					
Cases/person-time at risk (years)	11/462	11/508	12/405		
HR (95%CI)	1 (ref)	0.92 (0.13-6.59)	0.27 (0.03-2.22)	0.208	

(Continued)	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
Current cigarette smokers					
1-<20 cigarettes/day					
Cases/person-time at risk (years)	53/2,018	47/1,514	30/1,190		
HR (95%CI)	1 (ref)	1.36 (0.70-2.64)	1.26 (0.58-2.74)	0.672	0.797
20+ cigarettes/day					
Cases/person-time at risk (years)	73/903	46/505	26/410		
HR (95%CI)	1 (ref)	1.50 (0.72-3.12)	1.09 (0.39-3.06)	0.893	

[a] Adjusted for age (years; continuous), cigarette smoking (frequency (n/day; continuous, centered) and duration (years; continuous, centered)), body mass index (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of lung cancer (yes, no), history of chronic bronchitis (yes, no), daily energy intake (kcal/day; continuous), alcohol consumption (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), and alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points)

relations in lighter smokers (1-20 cigarettes/day) were also observed in the NIH-AARP [12]. A possible explanation for these observations is the high amount of antioxidants in nuts, e.g. vitamin E, selenium, proanthocyanidins, flavonoids, resveratrol, and carotenoids [29, 30]. In a crossover trial, almond supplementation significantly reduced biomarkers of oxidative stress and increased antioxidant defenses in men smoking 5-20 cigarettes/day [31]. The large amount of reactive oxygen species generated by heavy smoking (20+ cigarettes/day) might exceed the antioxidant capacity of nuts, possibly explaining the weaker associations in this subgroup. Nonetheless, no studies have investigated the relation of nut intake with oxidation status in heavy smokers. Moreover, in vivo studies, animal experiments, and human randomized controlled trials have not consistently observed beneficial effects of nut consumption on antioxidant status [30].

Another hypothesized mechanism relates to the anti-inflammatory and immune modulating effects of nuts, by compounds like α-linolenic acid, magnesium, L-arginine, flavonoids, and resveratrol [5, 25, 32]. Nevertheless, a recent meta-analysis of 23 randomized clinical trials found that, out of six inflammatory markers, nut consumption only significantly reduced the levels of intercellular adhesion molecule-1 [32]. Other potential mechanisms relate to the reduction of tumor initiation or promotion, regulation of DNA damage repair, metabolic enzyme activity, and hormonal mechanisms [5, 33]. Because the exact biological mechanism remains unclear, further research is warranted.

The (nonsignificant) inverse relations between nut intake and lung cancer risk were somewhat stronger for tree nut intake than for peanut intake, whereas no relations were observed for peanut butter intake. One possible explanation for these differences is the different nutrient composition of the nut types: peanuts, which are botanically legumes, contain less total fat than almonds, hazelnuts, and walnuts, whereas the amount of saturated fatty acids, proteins, carbohydrates, folate, and phytosterols is higher [34, 35]. Almonds, cashew nuts, and hazelnuts contain more monounsaturated fatty acids than peanuts, and walnuts contain more polyunsaturated fatty acids [35]. Moreover, almonds are better sources of fiber, magnesium, and calcium than peanuts [35]. Peanut butter that was sold

Table 5. Small cell carcinoma risk according to total nut intake in men, in multivariable-adjusted analyses[a], in strata of smoking status and smoking frequency; the Netherlands Cohort Study, 1986-2006

	Total nut consumption (g/day) per 5 g/day increment	$P_{interaction}$
Small cell carcinoma		
Men		
Smoking status		
Never		
Cases/person-time at risk (years)	13/4,345	
HR (95%CI)	0.45 (0.10-1.93)	0.574
Former		
Cases/person-time at risk (years)	110/15,791	
HR (95%CI)	0.88 (0.79-0.99)	
Current		
Cases/person-time at risk (years)	272/9,069	
HR (95%CI)	0.93 (0.87-1.00)	
Smoking frequency		
Former cigarette smokers		
1-<20 cigarettes/day		
Cases/person-time at risk (years)	31/8,647	
HR (95%CI)	1.00 (0.86-1.15)	0.075
20+ cigarettes/day		
Cases/person-time at risk (years)	79/7,145	
HR (95%CI)	0.82 (0.69-0.98)	
Current cigarette smokers		
1-<20 cigarettes/day		
Cases/person-time at risk (years)	113/5,318	
HR (95%CI)	0.96 (0.86-1.06)	0.332
20+ cigarettes/day		
Cases/person-time at risk (years)	159/3,751	
HR (95%CI)	0.90 (0.80-1.01)	

[a] Adjusted for age (years; continuous), cigarette smoking (frequency (n/day; continuous, centered) and duration (years; continuous, centered)), body mass index (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of lung cancer (yes, no), history of chronic bronchitis (yes, no), daily energy intake (kcal/day; continuous), alcohol consumption (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), and alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points)

in the Netherlands in 1986 contained more vitamin B6, sodium, and partially hydrogenated fatty acids, and less niacin than peanuts [34]. However, because the exact mechanism by which nuts might reduce the risk of lung cancer is unclear, we can only speculate about the differences between the observed relations for tree nuts, peanuts, and peanut butter. Furthermore, the food frequency questionnaire did not include questions about the consumption of specific tree nut subtypes. According to FAO trade data, tree nut subtypes that were imported into the Netherlands in 1986 included almonds, hazelnuts, walnuts, and cashew nuts [36]. These trade data give an indication of the tree nut subtypes that were available in the Netherlands at that time. Because the nutrient composition differs between tree nut subtypes [34], we recommend to further investigate their differential effects on

lung cancer risk in future studies.

The prospective design of the NLCS and its long and complete follow-up make information and selection bias unlikely. The large number of cases enabled us to stratify the results by the four lung cancer subtypes, by smoking characteristics, and by sex. Moreover, we were able to distinguish between tree nut, peanut, and peanut butter intake. Another advantage is the relatively high average nut intake in our study compared to most other cohort studies which investigated the relation between nut intake and lung cancer risk [11, 12]. Potential weaknesses include the single exposure measurement at baseline and possible measurement error, which may have attenuated our results. Residual confounding might be another limitation, although we extensively adjusted for potential confounders.

In conclusion, our cohort study showed that increased total nut, tree nut, and peanut intake is related to a significantly reduced risk of small cell carcinoma in men, after controlling for detailed smoking habits. Inverse, but nonsignificant associations were also seen for total lung cancer and the other histologic lung cancer subtypes in men. Nut intake was not related to lung cancer risk in women, and no significant associations were found for peanut butter intake in both sexes. Based on the overall body of evidence from the few prospective cohort studies investigating the relation between nut consumption and lung cancer risk, nut intake might be associated with a reduced risk of lung cancer, in particular the small cell carcinoma subtype. Cigarette smoking possibly modifies this relation. However, the evidence regarding this topic is still very limited and more prospective evidence is required before firm conclusions can be drawn. In addition, the exact biological mechanism that explains the observed relations has to be elucidated yet.

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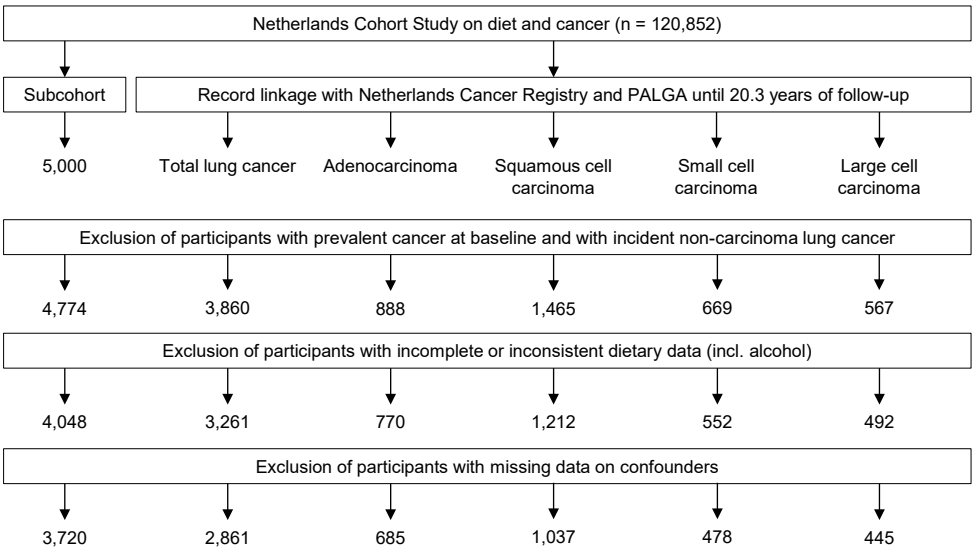
Supplementary Table S1. Multivariable-adjusted hazard ratios[a] (and 95% confidence intervals) of total lung cancer, comparing total nut intake categories of 0.1-<5 and 5+ g/day to 0 g/day, in subgroups of potential effect modifiers

	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
Overall					
Men					
Cases/person-time at risk (years)	892/8,663	731/9,588	777/10,877		
HR (95%CI)	1 (ref)	0.88 (0.72-1.07)	0.86 (0.71-1.05)	0.228	0.771
Women					
Cases/person-time at risk (years)	192/13,050	145/12,154	99/7,990		
HR (95%CI)	1 (ref)	1.07 (0.78-1.45)	1.02 (0.70-1.48)	0.976	
Body mass index					
Men					
18.5-<25 kg/m^2					
Cases/person-time at risk (years)	483/4,371	391/5,454	425/5,892		
HR (95%CI)	1 (ref)	0.81 (0.61-1.06)	0.86 (0.65-1.14)	0.543	0.702
25-<30 kg/m^2					
Cases/person-time at risk (years)	372/3,849	314/3,804	323/4,730		
HR (95%CI)	1 (ref)	1.00 (0.74-1.37)	0.81 (0.59-1.10)	0.120	
≥30 kg/m^2					
Cases/person-time at risk (years)	37/444	26/330	29/255		
HR (95%CI)	1 (ref)	0.72 (0.17-3.11)	1.58 (0.35-7.09)	0.406	
Women					
18.5-<25 kg/m^2					
Cases/person-time at risk (years)	112/6,755	87/6,462	68/5,201		
HR (95%CI)	1 (ref)	1.32 (0.87-2.02)	1.17 (0.71-1.94)	0.714	0.802
25-<30 kg/m^2					
Cases/person-time at risk (years)	65/4,822	51/4,732	25/2,295		
HR (95%CI)	1 (ref)	0.88 (0.51-1.53)	0.83 (0.43-1.62)	0.624	
≥30 kg/m^2					
Cases/person-time at risk (years)	15/1,472	7/960	6/494		
HR (95%CI)	1 (ref)	1.24 (0.35-4.45)	1.55 (0.28-8.52)	0.630	
Nonoccupational physical activity					
Men					
≤30 min/day					
Cases/person-time at risk (years)	185/1,341	152/1,551	142/1,807		
HR (95%CI)	1 (ref)	0.99 (0.62-1.60)	0.89 (0.54-1.45)	0.591	0.714
>30-≤60 min/day					
Cases/person-time at risk (years)	255/3,081	225/2,818	257/3,440		
HR (95%CI)	1 (ref)	1.07 (0.74-1.55)	1.01 (0.70-1.46)	0.962	
>60-≤90 min/day					
Cases/person-time at risk (years)	181/1,475	133/1,958	144/2,345		
HR (95%CI)	1 (ref)	0.75 (0.45-1.23)	0.62 (0.37-1.04)	0.094	
>90 min/day					
Cases/person-time at risk (years)	271/2,767	221/3,260	234/3,284		
HR (95%CI)	1 (ref)	0.78 (0.55-1.09)	0.83 (0.58-1.19)	0.562	
Women					
≤30 min/day					
Cases/person-time at risk (years)	62/3,424	40/2,478	32/1,391		
HR (95%CI)	1 (ref)	1.18 (0.61-2.27)	1.38 (0.60-3.14)	0.468	0.427

(Continued)	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
>30-≤60 min/day					
Cases/person-time at risk (years)	53/4,046	40/3,831	31/2,747		
HR (95%CI)	1 (ref)	0.91 (0.49-1.68)	1.03 (0.50-2.09)	0.888	
>60-≤90 min/day					
Cases/person-time at risk (years)	33/2,902	32/3,015	17/1,937		
HR (95%CI)	1 (ref)	1.06 (0.54-2.08)	0.65 (0.30-1.42)	0.225	
>90 min/day					
Cases/person-time at risk (years)	44/2,678	33/2,829	19/1,916		
HR (95%CI)	1 (ref)	0.62 (0.29-1.34)	0.40 (0.14-1.18)	0.153	
Alcohol intake					
Men					
0.0 g/day					
Cases/person-time at risk (years)	151/2,119	74/1,188	55/754		
HR (95%CI)	1 (ref)	1.25 (0.72-2.15)	1.42 (0.75-2.68)	0.295	0.205
0.1-<15 g/day					
Cases/person-time at risk (years)	334/3,694	331/5,294	314/4,907		
HR (95%CI)	1 (ref)	0.82 (0.61-1.11)	0.91 (0.67-1.24)	0.859	
≥15 g/day					
Cases/person-time at risk (years)	407/2,850	326/3,105	408/5,216		
HR (95%CI)	1 (ref)	0.84 (0.61-1.14)	0.72 (0.54-0.96)	0.037	
Women					
0.0 g/day					
Cases/person-time at risk (years)	81/5,506	36/3,381	28/1,459		
HR (95%CI)	1 (ref)	0.90 (0.52-1.58)	1.43 (0.69-2.97)	0.329	0.224
0.1-<15 g/day					
Cases/person-time at risk (years)	77/6,082	70/7,288	46/5,181		
HR (95%CI)	1 (ref)	0.82 (0.52-1.28)	0.80 (0.48-1.32)	0.484	
≥15 g/day					
Cases/person-time at risk (years)	34/1,462	39/1,485	25/1,349		
HR (95%CI)	1 (ref)	1.98 (0.89-4.39)	0.96 (0.33-2.76)	0.567	
Educational level					
Men					
Low					
Cases/person-time at risk (years)	526/4,326	389/4,254	358/3,667		
HR (95%CI)	1 (ref)	0.87 (0.66-1.15)	0.93 (0.69-1.24)	0.769	0.644
Medium					
Cases/person-time at risk (years)	271/2,833	255/3,430	273/4,398		
HR (95%CI)	1 (ref)	0.83 (0.59-1.18)	0.75 (0.53-1.06)	0.154	
High					
Cases/person-time at risk (years)	95/1,504	87/1,904	146/2,812		
HR (95%CI)	1 (ref)	0.90 (0.54-1.50)	1.04 (0.64-1.67)	0.714	
Women					
Low					
Cases/person-time at risk (years)	120/8,124	74/6,292	47/3,646		
HR (95%CI)	1 (ref)	1.11 (0.73-1.68)	0.98 (0.59-1.63)	0.883	0.577
Medium					
Cases/person-time at risk (years)	56/4,156	60/4,539	39/3,294		
HR (95%CI)	1 (ref)	1.27 (0.74-2.20)	1.07 (0.54-2.11)	0.956	
High					
Cases/person-time at risk (years)	16/770	11/1,322	13/1,050		
HR (95%CI)	1 (ref)	1.12 (0.25-5.05)	2.19 (0.48-10.01)	0.226	

(Continued)	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
aMed score excluding nuts and alcohol					
Men					
0-2					
Cases/person-time at risk (years)	330/2,949	245/2,609	217/2,686		
HR (95%CI)	1 (ref)	1.06 (0.73-1.53)	1.09 (0.75-1.58)	0.669	0.497
3-4					
Cases/person-time at risk (years)	434/4,139	360/4,662	380/5,390		
HR (95%CI)	1 (ref)	0.82 (0.62-1.09)	0.80 (0.60-1.07)	0.230	
5-7					
Cases/person-time at risk (years)	128/1,576	126/2,317	180/2,800		
HR (95%CI)	1 (ref)	0.59 (0.36-0.97)	0.72 (0.45-1.17)	0.679	
Women					
0-2					
Cases/person-time at risk (years)	65/3,797	51/2,715	31/1,723		
HR (95%CI)	1 (ref)	1.49 (0.78-2.83)	1.97 (0.91-4.25)	0.098	0.145
3-4					
Cases/person-time at risk (years)	100/6,554	63/5,962	46/3,845		
HR (95%CI)	1 (ref)	0.70 (0.45-1.08)	0.65 (0.38-1.10)	0.181	
5-7					
Cases/person-time at risk (years)	27/2,698	31/3,477	22/2,421		
HR (95%CI)	1 (ref)	1.85 (0.85-4.02)	1.54 (0.60-3.92)	0.603	
Family history of lung cancer					
Men					
No					
Cases/person-time at risk (years)	777/7,913	642/8,743	672/9,788		
HR (95%CI)	1 (ref)	0.88 (0.72-1.09)	0.86 (0.70-1.06)	0.230	0.953
Yes					
Cases/person-time at risk (years)	115/751	89/845	105/1,089		
HR (95%CI)	1 (ref)	0.72 (0.36-1.44)	0.90 (0.48-1.70)	0.998	
Women					
No					
Cases/person-time at risk (years)	169/11,633	124/10,918	85/7,203		
HR (95%CI)	1 (ref)	1.06 (0.76-1.47)	1.08 (0.73-1.59)	0.747	0.893
Yes					
Cases/person-time at risk (years)	23/1,417	21/1,236	14/787		
HR (95%CI)	1 (ref)	1.71 (0.53-5.46)	1.25 (0.34-4.62)	0.991	

[a] Adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered) and duration (years; continuous, centered)), body mass index (18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of lung cancer (yes, no), history of chronic bronchitis (yes, no), daily energy intake (kcal/day; continuous), alcohol consumption (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), and alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points)



Supplementary Figure S1. Flow diagram of the number of subcohort members and lung cancer cases who were included in the case-cohort analyses

Chapter 6

Tree nut, peanut, and peanut butter intake and the risk of postmenopausal breast cancer: The Netherlands Cohort Study

Piet A. van den Brandt and Lisette Nieuwenhuis

Cancer Causes Control. 2018; 29: 63-75

Abstract

Purporse: Nut intake has been associated with reduced mortality and risk of cardiovascular diseases, but there is only limited evidence on cancer. We investigated the relationship between nut intake and risk of postmenopausal breast cancer, and estrogen/progesterone receptor (ER/PR) subtypes.

Methods: In the Netherlands Cohort Study, 62,573 women aged 55-69 years provided information on dietary and lifestyle habits in 1986. After 20.3 years of follow-up, 2,321 incident breast cancer cases and 1,665 subcohort members were eligible for multivariate case-cohort analyses.

Results: Total nut intake was significantly inversely related to ER negative (ER-) breast cancer risk, with HR 0.55 (95% CI 0.33-0.93) for those consuming at least 10 g nuts/day versus nonconsumers (*p* trend = 0.025). There were no significant inverse associations with ER+ or total breast cancer. While there was no variation between PR subtypes, the ER-PR- subtype was also significantly inversely associated with nut intake, with HR 0.53 (95% CI 0.29-0.99), *p* trend = 0.037. Intake of peanuts and tree nuts separately was also inversely related to ER- breast cancer subtypes, while no associations were found with peanut butter intake.

Conclusion: Our findings suggest an inverse association between nut intake and ER- breast cancer, and no association with total or hormone receptor-positive subtypes.

Introduction

Nut intake has been associated with reduced risk of non-communicable diseases such as cardiovascular diseases (CVD) and diabetes (1). Apart from CVD, interest is growing in mortality and other health effects as well, stimulated by the PREDIMED trial showing effects of Mediterranean diet supplemented with mixed nuts or olive oil on CVD and depression (2). In several cohort studies, nut intake has been associated with lower total mortality and cancer mortality, e.g. (3-5), but few studies have been done on nut intake and risk of cancer. Also, little is known on differences between tree nuts and peanuts, and whether peanut butter shows similar associations with risk as peanuts. In addition, dose-response relationships remain unclear.

For breast cancer, two cohort studies have investigated the association between nut intake and breast cancer risk (6, 7); both found no association with overall breast cancer risk. However, no distinction was made between tree nuts, peanuts and peanut butter (these were grouped). One recent large population-based case-control study showed a significant inverse association between total nut intake and breast cancer (OR 0.76), with a stronger association for postmenopausal than premenopausal breast cancer (8). Recent evidence from a randomized controlled trial on primary prevention of cardiovascular diseases indicated a potentially strong protective effect of Mediterranean diet supplemented with nuts on the risk of postmenopausal breast cancer in Spain; however, this was not significant, probably due to small number of cases during short follow-up (9). For proliferative benign breast disease, a cohort study showed that two or more servings of nuts per week during adolescence was inversely associated with 36% lower risk of benign breast disease, compared with an intake of less than one serving per month. Statistically significant inverse associations were also observed for peanut intake alone (10).

It is important to distinguish between pre- and postmenopausal breast cancer, as well as estrogen/progesterone receptor (ER/PR) subtypes, because of differences in etiology.

We investigated the associations between intakes of total nuts, tree nuts, peanuts and peanut butter, and postmenopausal breast cancer risk, overall and stratified by hormone receptor status, in the Netherlands Cohort Study (NLCS). We recently found an inverse association between Mediterranean diet (MD) adherence and ER- breast cancer in the NLCS (11), in which nuts seemed to play a dominant role. Here, we further investigated this and evaluated tree nuts, peanuts, and peanut butter separately, while controlling for MD adherence.

Materials and methods

Study design and cancer follow-up

The NLCS started in September 1986 and the female part included 62,573 women aged 55-69 years (12). At baseline, participants completed a mailed, self-administered questionnaire

on cancer risk factors. The NLCS study was approved by institutional review boards from Maastricht University and the Netherlands Organization for Applied Scientific Research. All cohort members consented to participation by completing the questionnaire. For data processing and analysis, the case-cohort method was used (13). Accumulated person-years in the cohort were estimated from a subcohort (*n* = 2589 women), randomly sampled from the cohort immediately after baseline. These subcohort members were actively followed up biennially for vital status information. The follow-up of the subcohort was 100% complete at 20.3 years of follow-up.

Follow-up for cancer incidence in the entire cohort was established by annual record linkage with the Netherlands Cancer Registry and PALGA, the nationwide Dutch Pathology Registry (14). Completeness of follow-up through record linkage with cancer registries and PALGA was estimated to be greater than 95% (15). After 20.3 years of follow-up (17 September 1986 until 1 January 2007), a total of 3,354 incident breast cancer cases (of whom 144 were also subcohort member) were detected among women. Cases and subcohort members were excluded if they reported a history of cancer (except skin cancer) at baseline and if their dietary data were incomplete or inconsistent (16). Figure S1 (Supplementary data) shows the selection and exclusion steps that resulted in the number of cases and female subcohort members that were included in the analysis. There were 1,665 subcohort members and 2,321 breast cancer cases available for analysis. As the with nested case-control study, the case-cohort study is also nested within a cohort with comparable efficiency gain. An additional advantage of the case-cohort design over the nested case-control design is that the selected subcohort can be used to study a range of disease endpoints (17).

Exposure assessment

The 11-page baseline questionnaire measured dietary intake, detailed smoking habits, anthropometry, physical activity, and other risk factors related to cancer (12). Habitual consumption of food and beverages during the year preceding baseline was assessed using a 150-item semi-quantitative food frequency questionnaire, which was validated against a 9-day diet record (16). Nut and peanut butter consumption was assessed by asking frequency and portion size of intake of 'peanuts', 'other nuts, mixed nuts', and 'peanut butter'. Frequency of consumption could range from 'never or less than 1x/month' to '6-7x/week'. In addition, participants could fill in the number of standard portion sizes they consumed per intake. For tree nuts and peanuts, a standard portion size was 28 grams. A standard portion size of peanut butter, a particularly popular spread in the Netherlands, was 15 grams per slice of bread. Consumption frequencies and portion sizes were multiplied to calculate mean daily intakes in grams. Total nut intake was calculated as the sum of peanuts and other nuts. Nutrient intakes were calculated using the computerized Dutch food composition table (18).

Statistical analysis

For the intakes of nuts and peanut butter, the mean (SD) values were calculated in the subcohort. The distribution of the subcohort members by nut intake level and various characteristics was examined by cross-tabulations and summary statistics.

The relationship between intake of nuts and breast cancer risk was evaluated using Cox proportional hazards models. It was verified that the proportional hazards assumption was not violated using Schoenfeld residuals (19) and -ln(-ln) survival plots. Standard errors were estimated using the robust Huber–White sandwich estimator to account for additional variance introduced by the subcohort sampling (20).

In age- and multivariable-adjusted survival analyses, total nut intake was evaluated and tested on categorical (0, 0.1-<5, 5-<10, 10+ g/day) and continuous scales. In multivariable analyses, hazard ratios (HRs) were corrected for potential confounders: age at baseline (55-59, 60-64, 65-69 years), cigarette smoking (status (never, former, current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)), body height (continuous, cm), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), highest level of education (primary school or lower vocational, secondary or medium vocational, and higher vocational or university), family history of breast cancer in mother or sisters (no, yes), history of benign breast disease (no, yes), age at menarche (≤12, 13-14, 15-16, >17 years), parity (nulliparous, 1-2, ≥3 children), age at first birth (<25, ≥25 years), age at menopause (<45, 45-49, 50-54, ≥55 years), oral contraceptive use (never, ever), postmenopausal hormone replacement therapy (never, ever), energy intake (continuous, kcal/day), alcohol intake (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day). Because we recently found an association between Mediterranean diet adherence and breast cancer risk (11), we additionally adjusted for adherence to the Mediterranean diet as measured with the alternate Mediterranean diet (aMED) score (21). Since nuts comprise one of the components of the aMED-score and because alcohol consumption is positively associated with breast cancer risk, an adapted version (excluding nuts and alcohol) was used here, which ranged from 0 (no adherence) to 7 (maximal adherence).

Linear trends between nut intake categories and breast cancer were evaluated with Wald tests, after fitting median values of nut consumption per intake category as continuous terms in the regression model. Median values were based on the distribution of the variables in the subcohort. Analyses were also done for peanuts and tree nuts separately, and peanut butter; because of lower numbers in the high intake categories, we used categories 0, 0.1-<5, 5+ g/day.

Besides overall postmenopausal breast cancer, we conducted these analyses for subtypes defined by hormone receptor status: ER+, ER-, PR+, PR-, ER+PR+, and ER-PR-. Differences in associations with nut intake between breast cancer subtypes were tested using a heterogeneity test (22), in which the standard error for the observed difference in rate ratios was estimated using a bootstrapping method developed for the case-cohort design (23).

To further investigate the dose-response relations between nut consumption and breast cancer risk, restricted cubic splines with three fixed knots (0, 5, and 10 g/day) were used to graphically present the dose-response curves without making a priori assumptions about their shapes. Wald tests were performed to evaluate the linearity of these relationships.

To evaluate potential residual confounding by breast cancer risk factors, and effect modification, analyses of nut intake and breast cancer were also conducted within strata of alcohol intake, BMI, physical activity, and family history of breast cancer, and adapted aMED-score. Interactions with these factors were tested using Wald tests and cross-product terms. In sensitivity analyses, we repeated analyses after excluding cancers (and person-years) occurring in the first two years of follow-up. Moreover, analyses of peanut butter consumption were repeated, restricted to respondents who had stated having had the same peanut butter intake during the five years before baseline. Unfortunately, these data were unavailable for total nut, tree nut, and peanut consumption.

Analyses were performed using Stata version 12; presented *p* values are two-sided, with $p<0.05$ considered as statistically significant.

Results

Mean total nut consumption (SD) in subcohort women was 4.3 (8.4) g/day; for tree nut, peanut, and peanut butter, these values were 1.0 (3.9), 3.3 (6.9), and 1.2 (3.6), respectively. Nut consumers were on average somewhat younger (Table 1), leaner, drank more alcohol, less often reported a positive family history of breast cancer, and were less often never smokers. They were higher educated, more often had a late age at birth of their first child, scored higher on the adapted aMED-score, and more often used oral contraceptives and postmenopausal HRT. Peanut butter intake was weakly positively associated with nut intake.

Peanut butter consumers were on average somewhat younger (Table 1), leaner, drank less alcohol, less often reported a history of benign breast disease or family history of breast cancer, and were more often never smokers, but were higher educated, more often had a late age at birth of first child, scored higher on the adapted aMED-score, and more often used oral contraceptives and postmenopausal HRT.

Table 2 shows results of age-adjusted and multivariable-adjusted analyses of the associations of total nut intake with total breast cancer risk, and risk of estrogen and progesterone receptor subtypes. Total nut intake was not associated with total breast cancer risk in categorical or continuous analyses. Compared to nonconsumers of nuts, the HR (95% CI) of breast cancer for those consuming at least 10 g nuts/day was 0.91 (0.72-1.14) (*p* trend = 0.625) in multivariable analyses. Additional analyses adjusting for peanut butter intake yielded similar results (data not shown). ER+ breast cancer also showed no associations, but ER- breast cancer was significantly inversely associated with total nut intake. Compared to nonconsumers of nuts, the HRs (95% CIs) of ER- breast cancer for those consuming 0.1-<5, 5-<10, or at least 10 g nuts/day were 0.78 (0.56-1.08), 0.60 (0.34-1.05), and 0.55 (0.33-0.93), respectively (*p* trend = 0.025). No clear associations were seen for PR subtypes. Risk of ER-PR- breast cancer was significantly inversely related to total nut intake in multivariable-adjusted analyses with a HR for those consuming at least 10 g nuts/day versus nonconsumers of 0.53 (95% CI 0.29-0.99) with *p* trend = 0.037. The ER+PR+ subtype

Table 1. Baseline characteristics (mean (SD), or percentage) according to category of total nut intake in subcohort women with complete dietary data, Netherlands Cohort Study

Characteristic	Total nut intake (g/day)				Peanut butter intake (g/day)		
	0 g/day (n = 821)	0.1-<5 (n = 735)	5-<10 (n = 225)	10+ g/d (n = 246)	0 g/day (n = 1493)	0.1-<5 (n = 351)	5+ g/d (n = 183)
Age (years)	62.2 (4.4)	61.1 (4.2)	60.1 (4.0)	60.6 (3.9)	61.6 (4.3)	60.9 (4.0)	60.7 (4.0)
Height (cm)	164.9 (6.5)	165.5 (5.8)	165.6 (6.0)	165.8 (6.2)	165.4 (6.1)	165.1 (6.0)	165.2 (6.6)
BMI (kg/m^2)	25.3 (3.8)	25.1 (3.4)	24.4 (3.1)	24.5 (3.3)	25.0 (3.6)	25.2 (3.4)	24.4 (3.2)
Physical activity (min/day)	62.9 (55.2)	65.8 (48.1)	71.7 (55.1)	60.8 (37.4)	62.3 (48.4)	72.8 (60.0)	68.3 (49.2)
Age at menarche (years)	13.7 (1.8)	13.6 (1.7)	13.7 (1.8)	13.6 (1.7)	13.6 (1.7)	13.7 (1.9)	13.8 (1.9)
Age at menopause (years)	48.7 (4.4)	48.7 (4.6)	49 (4.4)	48.9 (4.3)	48.7 (4.5)	48.6 (4.4)	49.6 (4.2)
Alcohol intake (g/day)	4.9 (9.5)	5.8 (8.8)	7.2 (9.3)	8.6 (10.9)	6.1 (9.7)	5.5 (9.1)	4.9 (7.9)
Peanut butter intake (g/day)	1.0 (3.5)	1.2 (3.7)	1.3 (3.5)	1.3 (3.3)			
Total nut intake (g/day)					4.1 (8.3)	5.2 (8.7)	4.9 (8.5)
Never smoker (%)	60.7	59.3	49.8	50.4	57.9	54.7	62.3
University or higher vocational education (%)	6.2	10.7	14.3	12.6	8.4	11.5	14.8
Nulliparous (%)	17.7	20.5	16.9	17.6	18.1	19.0	22.0
Age at first birth $\geq$ 30 years (% of parous)	22.8	19.5	26.6	26.7	22.4	21.3	25.9
Ever used oral contraceptives (%)	20.6	24.9	35.6	33.2	24.3	27.8	29.5
Ever used hormone replacement therapy (%)	12.2	13.8	13.3	16.4	13.8	13.5	9.7
Family history breast cancer (%)	8.4	10.2	6.7	7.7	9.1	9.1	5.5
History benign breast disease (%)	6.8	9.4	8.4	6.5	8.5	6.3	6.0
aMEDr[1] score (excluding nuts) 5-7 pts (%)	20.5	27.3	26.7	32.1	23.4	27.9	33.3

[1] aMEDr: alternate Mediterranean Diet Score excluding alcohol

showed no significant association with total nut intake (Table 2). Heterogeneity tests across subtypes using bootstrapping were not significant (data not shown). Analyses for the ER+/PR- subtype closely resembled those of ER+ subtypes and was therefore not reported; the number of ER-/PR+ cases was too small for separate analyses.

Table 3 shows results of multivariable analyses for intake of peanuts, tree nuts, and peanut butter separately. For peanuts and tree nuts, the pattern of associations resembled that for total nut intake, i.e., inverse associations were observed for ER- and ER-PR- subtypes, albeit nonsignificant. For peanuts, the HR (95% CI) for ER- breast cancer, comparing for 5+ versus 0 g /day was 0.63 (0.40-1.01), with *p* trend = 0.059. For tree nuts, this was 0.47 (0.21-1.09) for the same contrast (*p* trend = 0.079). Similarly, for ER-PR- breast cancer the HR (95% CI) was 0.46 (0.17-1.23), (*p* trend = 0.124), when comparing for 5+ versus 0 g tree nuts/day. Peanut butter intake was not associated with total breast cancer risk or its subtypes (Table 3). Excluding of the first two years of follow-up, or limiting the peanut butter analyses to those with stable intake over the past five years, did not materially change the results (data not shown).

Restricted cubic spline curves for the HR according to intake of total nuts, peanuts, tree nuts, and peanut butter are shown in separate panels in Figure 1 for total breast cancer, and in Figure 2 for ER- breast cancer. None of the tests for nonlinearity were statistically significant (*p* values are shown in Figure legends). However, for total nuts and ER- breast cancer, it was borderline significant, and the exposure-response curves for total nuts, peanuts, and tree nuts show a clear leveling off with intake levels above 10 g/day. Therefore, effect modification and subgroup analyses were conducted using a categorical variable for nut intake: 0, 0.1-<5, and 5+ g/day (combined upper category was needed because of sample size).

In Figure 3, HRs and 95% CIs for breast cancer and ER subtypes are presented for the two categories of total nut intake (0.1-<5 and 5+ g/day) versus 0 g/day, overall, and in subgroups of potential effect modifiers: alcohol intake, BMI, physical activity, and adapted aMED-score (excluding alcohol and nuts). For total breast cancer, no association with nut intake was seen in most subgroups. For total and ER+ breast cancer, there was significant interaction between nut intake and BMI-level, with decreased HRs observed in the subgroup BMI 18.5-<25 kg/m^2 (Total breast cancer: HR 0.79 (0.61-1.02); ER+: HR 0.84 (0.61-1.15)), and (significantly) increased HRs observed for BMI ≥25 kg/m^2 (Total: HR 1.32 (0.96-1.81); ER+: HR 1.50 (1.03-2.18)). For the ER- subtype, inverse associations were seen in most subgroups, and there was no significant interaction with these covariables. Possible interactions with age at baseline (55-59, 60-64, 65-69 years), smoking status (never, ex, current), and family history of breast cancer (no, yes) were also investigated, but were all nonsignificant.

Discussion

In this large prospective study, we found a statistically significant inverse association between nut intake and risk of estrogen receptor-negative postmenopausal breast cancer.

Table 2. Hazard Ratio of breast cancer and subtypes, according to total nut intake in multivariable-adjusted[1] analyses, Netherlands Cohort Study

	Total nut intake (g/day) (median)						
	0 g/d (0)	0.1-<5 g/d (2.1)	5-<10 g/d (7.8)	10+ g/d (15.7)	*p* trend	Continuous per 10 g/d	*p* non-linear
Total breast cancer							
No. of cases	935	844	251	291		2,321	
Person-years in subcohort	11,322	10,646	3,067	3,897		28,932	
Age-adjusted HR	1	0.97	1.02	0.91	0.471	1.01	
(95% CI)		(0.83 - 1.12)	(0.81 - 1.27)	(0.74 - 1.12)		(0.94 - 1.09)	
Multivariable-adjusted HR	1	0.94	1.05	0.91	0.625	1.02	0.343
(95% CI)		(0.80 - 1.10)	(0.82 - 1.34)	(0.72 - 1.14)		(0.94 - 1.11)	
ER+ breast cancer							
No. of cases	436	415	119	151		1,121	
Age-adjusted HR	1	1.03	1.04	1.01	0.978	1.03	
(95% CI)		(0.86 - 1.23)	(0.79 - 1.36)	(0.79 - 1.29)		(0.95 - 1.13)	
Multivariable-adjusted HR	1	1.02	1.10	1.07	0.589	1.06	0.429
(95% CI)		(0.84 - 1.24)	(0.82 - 1.48)	(0.81 - 1.41)		(0.96 - 1.17)	
ER- breast cancer							
No. of cases	117	87	20	24		248	
Age-adjusted HR	1	0.79	0.64	0.58	0.022	0.84	
(95% CI)		(0.58 - 1.07)	(0.38 - 1.07)	(0.36 - 0.93)		(0.66 - 1.06)	
Multivariable-adjusted HR	1	0.78	0.60	0.55	0.025	0.83	0.054
(95% CI)		(0.56 - 1.08)	(0.34 - 1.05)	(0.33 - 0.93)		(0.64 - 1.07)	
PR+ breast cancer							
No. of cases	273	277	69	84		703	
Age-adjusted HR	1	1.10	0.97	0.89	0.305	0.99	
(95% CI)		(0.90 - 1.35)	(0.70 - 1.33)	(0.67 - 1.20)		(0.89 - 1.10)	
Multivariable-adjusted HR	1	1.06	0.97	0.94	0.576	1.01	0.278
(95% CI)		(0.85 - 1.33)	(0.68 - 1.40)	(0.68 - 1.30)		(0.89 - 1.15)	
PR- breast cancer							
No. of cases	158	128	40	49		375	
Age-adjusted HR	1	0.85	0.92	0.86	0.557	1.02	
(95% CI)		(0.66 - 1.11)	(0.62 - 1.36)	(0.60 - 1.24)		(0.89 - 1.17)	
Multivariable-adjusted HR	1	0.89	0.95	0.87	0.637	1.02	0.240
(95% CI)		(0.67 - 1.18)	(0.62 - 1.47)	(0.58 - 1.31)		(0.88 - 1.19)	
ER+PR+ breast cancer							
No. of cases	263	272	68	82		685	
Age-adjusted HR	1	1.12	0.99	0.91	0.351	0.99	
(95% CI)		(0.91 - 1.38)	(0.72 - 1.37)	(0.67 - 1.22)		(0.89 - 1.11)	
Multivariable-adjusted HR	1	1.08	1.00	0.95	0.596	1.01	0.344
(95% CI)		(0.86 - 1.36)	(0.69 - 1.43)	(0.68 - 1.31)		(0.89 - 1.15)	
ER-PR- breast cancer							
No. of cases	76	63	14	17		170	
Age-adjusted HR	1	0.86	0.65	0.61	0.064	0.89	
(95% CI)		(0.60 - 1.24)	(0.35 - 1.21)	(0.35 - 1.07)		(0.68 - 1.16)	
Multivariable-adjusted HR	1	0.86	0.61	0.53	0.037	0.85	0.100
(95% CI)		(0.58 - 1.27)	(0.31 - 1.18)	(0.29 - 0.99)		(0.64 - 1.14)	

[1] Multivariable analyses were adjusted for the following: age at baseline (55-59, 60-64, 65-69 years), cigarette smoking (status (never, former, current), frequency (number of cigarettes per day;

continuous, centered), duration (number of years; continuous, centered)), body height (continuous, cm), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), highest level of education (primary school or lower vocational, secondary or medium vocational, and higher vocational or university), family history of breast cancer in mother or sisters (no, yes), history of benign breast disease (no, yes), age at menarche (<12, 13-14, 15-16, ≥17 years), parity (nulliparous, 1-2, ≥3 children), age at first birth (<25, ≥25 years), age at menopause (<45, 45-49, 50-54, ≥55 years), oral contraceptive use (never, ever), postmenopausal HRT (never, ever), energy intake (continuous, kcal/day), alcohol intake (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), alternate Mediterranean Diet Score excluding alcohol and nuts (0-2, 3-4, 5-7 pts)

There were no significant inverse associations with ER+ or total breast cancer risk. When comparing those consuming 10+ g nuts/day to nonconsumers, the HR for ER- breast cancer was 0.55, while it was 0.91 for total breast cancer and 1.07 for ER+ breast cancer. While there was no variation between PR subtypes, the ER-PR- subtype was also significantly inversely associated with nut intake. There was no statistical evidence of nonlinear dose-response relationships, but this nonlinearity test was borderline significant for ER- breast cancer, where the exposure-response curves show a clear leveling off with intake levels above 10 g/day. Intake of peanuts and tree nuts separately was also inversely related to ER- breast cancer subtypes, while no associations were found with peanut butter intake. There was significant interaction between total nut intake and BMI for total breast cancer and ER+ breast cancer risk.

The PREDIMED randomized controlled trial in Spain investigated whether following a Mediterranean diet supplemented with nuts compared to a control diet in which it was advised to decrease dietary fat reduced the risk of breast cancer (9). After a median follow-up of 4.8 years with 35 incident breast cancer cases, they found a RR of 0.59 (95% CI 0.26-1.35). Although this association was not significant, the HR was rather low and power was probably insufficient given the low number of cases. Since diets were analyzed and no direct comparisons between consumption of individual food items in both groups were made, it is unclear what the effect of nut consumption alone would be.

In two cohort studies, the relationship between nut consumption and risk of breast cancer was studied. Sonestedt et al. (6) in Sweden did not find a relation between nut intake and risk of breast cancer (HR for median consumption of 6 g/day vs. nonconsumers = 0.98, 95% CI 0.75-1.27). Also, no relation was found when stratifying on ER status (6). In the Nurses' Health Study II, no association was observed between the number of servings of peanuts, peanut butter, and other nuts per day in young adulthood and risk of breast cancer in both premenopausal and postmenopausal women (7).

Besides these cohort studies, six case-control studies on this topic were identified. A case-control study in Argentina found non-significantly increased breast cancer risks with higher nut (peanut and walnut) consumption when comparing cases to both hospital and neighborhood controls (24). An Italian case-control study found a significantly inverse association between seed oil consumption, including peanut oil, and breast cancer risk in

both pre- and postmenopausal women (25). In Canada, a significantly inverse relation was found between consumption frequency of total nuts during adolescence and breast cancer risk, which was mainly observed for postmenopausal breast cancer (8). In Mexico, risk of breast cancer was significantly inversely related to the consumption frequency of peanuts, walnuts, and almonds (26). Studies in Iran and the Central African Republic showed positive associations between (ground)nut intake and breast cancer, but no confounder adjustment was made (27, 28).

Two cohort studies were performed on nut intake and risk of benign breast disease, both in the US. Su et al. (10) found a statistically significant decreased risk of proliferative BBD when consuming nuts more often as adolescent (RR for ≥2/week vs. <1/month = 0.64, 95% CI 0.48-0.85), but this relation was not found for peanut butter consumption (10). An inverse association between nut consumption in adolescence and subsequent BBD risk was also observed by Berkey (29).

Thus, the literature is mixed with null results in two cohort studies and inverse and positive associations in case-control studies (which may be linked to storage conditions of nuts in developing countries), but very few studies have investigated hormone receptor subtypes.

We also found no association with total breast cancer, but did find an inverse association with ER- subtypes. This needs to be confirmed in other large cohort studies and trials, with analyses per receptor subtype. We did not have data on nut intake in adolescence or early adulthood, which might be etiologically relevant; future studies might also focus on that.

In the NLCS, we earlier reported inverse associations with MD adherence in the ER- subtypes (11). In the current effect-modification analysis, inverse associations with ER- breast cancer were seen in every subgroup of aMED excluding nuts (and alcohol), with the strongest inverse association found in those who had the lowest MD adherence score. This suggests an independent association between ER- breast cancer risk and nuts, apart from other MD-components. Nuts are a rich source of nutrients and energy, for example mono- and polyunsaturated fatty acids, protein, fiber, vitamins (e.g., various B-vitamins and vitamin E), minerals (e.g., magnesium, selenium), antioxidants, and phytochemicals like phenolic compounds and phytosterols (30), although the concentrations can vary among the different sorts of nuts (31). The potential mechanisms of action of these components of nuts in the prevention of cancer have been investigated, but not in great detail. Some of them are related to antioxidant activity, the regulation of cell differentiation and proliferation, the reduction of tumor initiation or promotion, the repair of DNA damage, anti-inflammatory responses, the regulation of immunological activity, the induction or inhibition of metabolic enzymes and hormonal mechanisms (32, 33).

Although nuts have a high fat content, they contain mainly monounsaturated (MUFA) or polyunsaturated fatty acids (PUFA), and are very low in saturated fat (34). When comparing peanuts to walnuts, it can be concluded that both are good sources of magnesium, MUFA, and PUFA, but that walnuts contain more alpha-linolenic acid; peanuts are richer in MUFA,

Table 3. Hazard Ratio of breast cancer and subtypes, according to intake of peanuts, tree nuts, and peanut butter, multivariable-adjusted[1] analyses, Netherlands Cohort Study

	Peanuts (g/day) (median)					Tree nuts (g/day) (median)					Peanut butter (g/day) (median)				
	0 g/d (0)	0.1-<5 g/d (2.0)	5+ g/d (10.7)	*p* trend	Continuous per 10 g/d	0 g/d (0)	0.1-<5 g/d (1.6)	5+ g/d (8.9)	*p* trend	Continuous per 10 g/d	0 g/d (0)	0.1-<5 g/d (1.2)	5+ g/d (5.3)	*p* trend	Continuous per 10 g/d
Total breast cancer															
No. of cases	1,087	855	379		2,321	1,649	566	106		2,321	1,702	416	203		2,321
Person-years in subcohort	13,457	10,600	4,875		28,932	20,351	6,880	1,701		28,932	20,932	5,245	2,754		28,932
Age-adjusted HR	1	1.01	0.97	0.771	1.04	1	1.02	0.77	0.114	0.94	1	0.98	0.90	0.364	0.86
(95% CI)		(0.87 - 1.16)	(0.81 - 1.17)		(0.95 - 1.14)		(0.88 - 1.19)	(0.58 - 1.03)		(0.80 - 1.11)		(0.83 - 1.16)	(0.72 - 1.13)		(0.71 - 1.05)
Multivariable-adjusted HR	1	1.00	0.98	0.809	1.04	1	1.00	0.80	0.191	0.95	1	1.05	1.01	0.965	0.95
(95% CI)		(0.86 - 1.17)	(0.79 - 1.20)		(0.94 - 1.14)		(0.84 - 1.18)	(0.58 - 1.10)		(0.79 - 1.16)		(0.88 - 1.26)	(0.79 - 1.29)		(0.77 - 1.16)
ER+ breast cancer															
No. of cases	526	408	187		1,121	784	286	51		1,121	827	200	94		1,121
Age-adjusted HR	1	1.00	0.99	0.949	1.06	1	1.08	0.78	0.269	0.97	1	0.97	0.85	0.270	0.84
(95% CI)		(0.84 - 1.19)	(0.79 - 1.24)		(0.95 - 1.18)		(0.90 - 1.30)	(0.55 - 1.12)		(0.81 - 1.17)		(0.79 - 1.20)	(0.65 - 1.13)		(0.65 - 1.07)
Multivariable-adjusted HR	1	1.01	1.02	0.860	1.07	1	1.11	0.88	0.661	1.03	1	1.04	0.98	0.921	0.93
(95% CI)		(0.84 - 1.22)	(0.80 - 1.32)		(0.95 - 1.21)		(0.91 - 1.36)	(0.60 - 1.30)		(0.85 - 1.25)		(0.84 - 1.30)	(0.73 - 1.33)		(0.73 - 1.20)
ER- breast cancer															
No. of cases	129	88	31		248	181	60	7		248	183	40	25		248
Age-adjusted HR	1	0.86	0.65	0.055	0.86	1	0.99	0.46	0.055	0.62	1	0.86	1.00	0.996	0.92
(95% CI)		(0.64 - 1.16)	(0.43 - 1.00)		(0.67 - 1.11)		(0.72 - 1.36)	(0.21 - 1.02)		(0.29 - 1.29)		(0.60 - 1.25)	(0.63 - 1.58)		(0.62 - 1.37)
Multivariable-adjusted HR	1	0.87	0.63	0.059	0.86	1	0.94	0.47	0.079	0.60	1	0.90	1.10	0.721	0.97
(95% CI)		(0.63 - 1.21)	(0.40 - 1.01)		(0.67 - 1.11)		(0.65 - 1.34)	(0.21 - 1.09)		(0.26 - 1.36)		(0.61 - 1.33)	(0.66 - 1.82)		(0.64 - 1.48)
PR+ breast cancer															
No. of cases	331	267	105		703	495	178	30		703	518	125	60		703
Age-adjusted HR	1	1.05	0.89	0.337	1.01	1	1.07	0.73	0.203	0.93	1	0.98	0.87	0.398	0.86
(95% CI)		(0.86 - 1.28)	(0.68 - 1.16)		(0.88 - 1.16)		(0.87 - 1.33)	(0.47 - 1.11)		(0.71 - 1.20)		(0.77 - 1.24)	(0.63 - 1.20)		(0.65 - 1.14)
Multivariable-adjusted HR	1	1.05	0.91	0.477	1.02	1	1.05	0.82	0.480	0.98	1	1.04	1.02	0.901	0.98
(95% CI)		(0.84 - 1.31)	(0.67 - 1.22)		(0.89 - 1.18)		(0.83 - 1.34)	(0.52 - 1.31)		(0.74 - 1.30)		(0.81 - 1.35)	(0.72 - 1.45)		(0.73 - 1.30)
PR- breast cancer															
No. of cases	182	128	65		375	262	96	17		375	279	66	30		375
Age-adjusted HR	1	0.87	0.95	0.851	1.05	1	1.08	0.76	0.400	0.91	1	0.92	0.78	0.236	0.75
(95% CI)		(0.68 - 1.13)	(0.69 - 1.32)		(0.90 - 1.23)		(0.83 - 1.41)	(0.45 - 1.31)		(0.67 - 1.24)		(0.68 - 1.25)	(0.51 - 1.18)		(0.51 - 1.12)

(Continued)	Peanuts (g/day) (median)					Tree nuts (g/day) (median)					Peanut butter (g/day) (median)				
	0 g/d (0)	0.1-<5 g/d (2.0)	5+ g/d (10.7)	*p* trend	Continuous per 10 g/d	0 g/d (0)	0.1-<5 g/d (1.6)	5+ g/d (8.9)	*p* trend	Continuous per 10 g/d	0 g/d (0)	0.1-<5 g/d (1.2)	5+ g/d (5.3)	*p* trend	Continuous per 10 g/d
Multivariable-adjusted HR	1	0.91	0.94	0.782	1.04	1	1.13	0.83	0.623	0.94	1	0.95	0.82	0.399	0.78
(95% CI)		(0.69 - 1.20)	(0.64 - 1.36)		(0.88 - 1.23)		(0.84 - 1.52)	(0.47 - 1.47)		(0.69 - 1.29)		(0.69 - 1.31)	(0.52 - 1.29)		(0.52 - 1.17)
ER+PR+ breast cancer															
No. of cases	321	261	103		685	481	175	29		685	500	125	60		685
Age-adjusted HR	1	1.06	0.90	0.387	1.01	1	1.09	0.72	0.210	0.93	1	1.01	0.90	0.537	0.88
(95% CI)		(0.87 - 1.30)	(0.69 - 1.18)		(0.88 - 1.16)		(0.88 - 1.34)	(0.47 - 1.12)		(0.72 - 1.21)		(0.80 - 1.29)	(0.65 - 1.25)		(0.67 - 1.17)
Multivariable-adjusted HR	1	1.06	0.91	0.505	1.02	1	1.06	0.81	0.458	0.98	1	1.08	1.06	0.750	1.00
(95% CI)		(0.85 - 1.32)	(0.68 - 1.24)		(0.88 - 1.18)		(0.83 - 1.35)	(0.51 - 1.30)		(0.74 - 1.30)		(0.84 - 1.40)	(0.74 - 1.51)		(0.75 - 1.34)
ER-PR- breast cancer															
No. of cases	86	61	23		170	122	43	5		170	122	29	19		170
Age-adjusted HR	1	0.87	0.70	0.167	0.92	1	1.04	0.48	0.124	0.69	1	0.92	1.11	0.687	1.07
(95% CI)		(0.61 - 1.24)	(0.43 - 1.14)		(0.70 - 1.21)		(0.72 - 1.51)	(0.19 - 1.20)		(0.30 - 1.60)		(0.60 - 1.41)	(0.66 - 1.87)		(0.70 - 1.62)
Multivariable-adjusted HR	1	0.88	0.63	0.105	0.88	1	0.98	0.46	0.124	0.65	1	0.99	1.20	0.537	1.14
(95% CI)		(0.60 - 1.28)	(0.36 - 1.09)		(0.66 - 1.17)		(0.63 - 1.51)	(0.17 - 1.23)		(0.25 - 1.66)		(0.63 - 1.56)	(0.67 - 2.14)		(0.72 - 1.80)

[1] Multivariable analyses were adjusted for the following: age at baseline (55-59, 60-64, 65-69 years), cigarette smoking (status (never, former, current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)), body height (continuous, cm), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), highest level of education (primary school or lower vocational, secondary or medium vocational, and higher vocational or university), family history of breast cancer in mother or sisters (no, yes), history of benign breast disease (no, yes), age at menarche (<12, 13-14, 15-16, ≥17 years), parity (nulliparous, 1-2, ≥3 children), age at first birth (<25, ≥25 years), age at menopause (<45, 45-49, 50-54, ≥55 years), oral contraceptive use (never, ever), postmenopausal HRT (never, ever), energy intake (continuous, kcal/day), alcohol intake (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), alternate Mediterranean Diet Score excluding alcohol and nuts (0-2, 3-4, 5-7 pts)

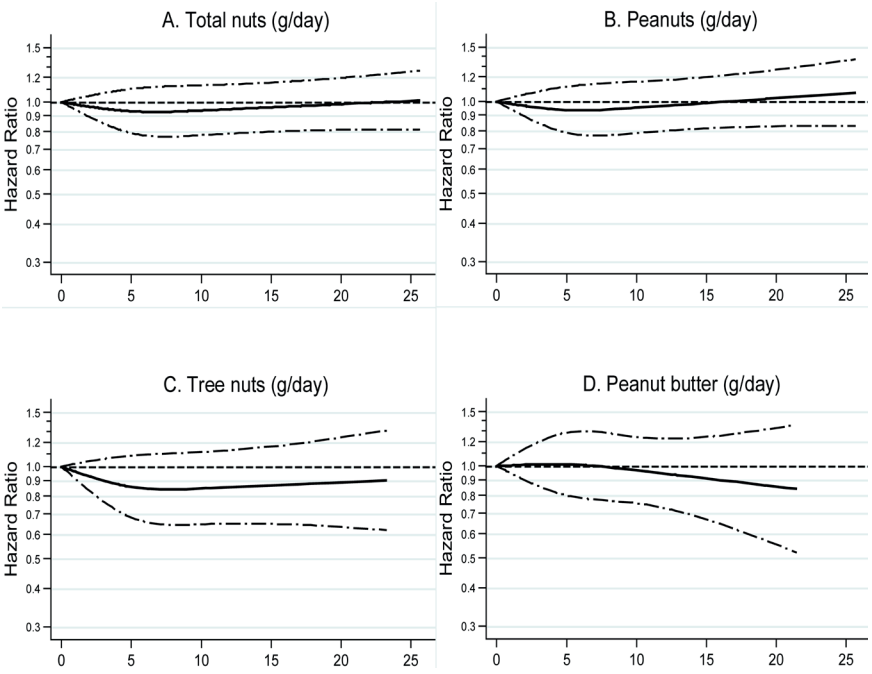


Figure 1. Nonparametric regression curves for the association between total breast cancer risk and A) total nut intake, B) peanuts, C) tree nuts and D) peanut butter intake (g/day). Multivariate HRs are calculated by restricted cubic spline regression (using 3 knots at 0, 5 and 10 g/day) adjusting for: age at baseline (55-59, 60-64, 65-69 years), cigarette smoking (status (never, former, current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)), body height (continuous, cm), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), highest level of education (primary school or lower vocational, secondary or medium vocational, and higher vocational or university), family history of breast cancer in mother or sisters (no, yes), history of benign breast disease (no, yes), age at menarche (≤12, 13-14, 15-16, ≥17 years), parity (nulliparous, 1-2, ≥3 children), age at first birth (<25, ≥25 years), age at menopause (<45, 45-49, 50-54, ≥55 years), oral contraceptive use (never, ever), postmenopausal hormone replacement therapy (never, ever), energy intake (continuous, kcal/day), alcohol intake (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), alternate Mediterranean Diet Score excluding alcohol and nuts (0-2, 3-4, 5-7 pts). To test for nonlinearity, the model including the linear and cubic spline terms was compared to the model with only the linear term using a Wald test. *P* values for nonlinearity were 0.343 for total nut intake, 0.347 for peanut intake, 0.212 for tree nuts, and 0.683 for peanut butter intake. Lines with dashes represent the 95% confidence intervals (CIs) for the fitted nonlinear trend (solid line).

protein, niacin, and potassium. The antioxidant capacity of walnuts is higher than peanuts or peanut butter (5). Peanuts, grapes, and red wine are primary sources of resveratrol. Resveratrol (a stilbene) has been shown to induce apoptosis, inhibit cell invasion and angiogenesis, and has been tested in in vivo models of breast, colorectal, liver, pancreatic, and prostate cancer. In addition, resveratrol and anacardic acid (a phenolic acid in cashews) seem to be able to counteract cancer-related epigenetic alterations (35). Epidemiologic studies and the PREDIMED trial have suggested an inverse association between nut consumption and inflammation (36). Finally, inositol polyphosphates (from peanuts) might be related to energy metabolism and cancer through the inhibition of the PI3K/Akt pathway (35).

The prospective design and high completeness of follow-up of the NLCS make information bias and selection bias unlikely. A potential weakness is the moderate proportion of breast cancer cases for whom ER/PR status was known. Breast cancer cases with known and unknown receptor status did not differ importantly according to baseline and tumor characteristics, making selection bias of the cases unlikely (data not shown). Although many possible confounders were taken into account, the possibility of confounding by unmeasured factors remains. The validation study of the food frequency questionnaire has shown that it performs relatively well (16), but measurement error may still have attenuated associations. The lack of possibilities to update dietary intake or other lifestyle data during follow-up may have resulted in some attenuated associations too.

In conclusion, our cohort study showed a statistically significant inverse association between total nut intake and risk of ER- breast cancer. There were no significant inverse associations with ER+ or total breast cancer risk. While there was no variation between PR subtypes, the ER-PR- subtype was also significantly inversely associated with nut intake. For ER- breast cancer, the exposure-response curves using restricted cubic splines showed a clear leveling off with intake levels above 10 g/day, but the nonlinearity test was not significant. Intake of peanuts and tree nuts separately was also inversely related to ER- breast cancer subtypes, while no associations were found with peanut butter intake.

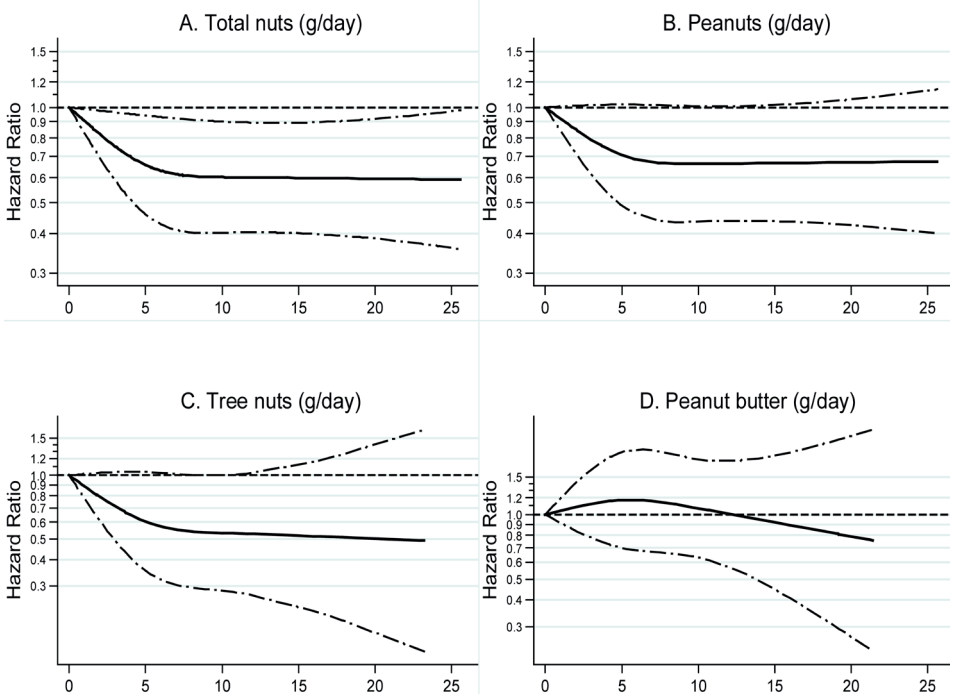


Figure 2. Nonparametric regression curves for the association between ER- breast cancer risk and A) total nut intake, B) peanuts, C) tree nuts and D) peanut butter intake (g/day). Multivariate HRs are calculated by restricted cubic spline regression (using 3 knots at 0, 5 and 10 g/day) adjusting for: age at baseline (55-59, 60-64, 65-69 years), cigarette smoking (status (never, former, current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)), body height (continuous, cm), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), highest level of education (primary school or lower vocational, secondary or medium vocational, and higher vocational or university), family history of breast cancer in mother or sisters (no, yes), history of benign breast disease (no, yes), age at menarche (≤12, 13-14, 15-16, ≥17 years), parity (nulliparous, 1-2, ≥3 children), age at first birth (<25, ≥25 years), age at menopause (<45, 45-49, 50-54, ≥55 years), oral contraceptive use (never, ever), postmenopausal hormone replacement therapy (never, ever), energy intake (continuous, kcal/day), alcohol intake (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), alternate Mediterranean Diet Score excluding alcohol and nuts (0-2, 3-4, 5-7 pts). To test for nonlinearity, the model including the linear and cubic spline terms was compared to the model with only the linear term using a Wald test. *P* values for nonlinearity were 0.054 for total nut intake, 0.099 for peanut intake, 0.202 for tree nuts, and 0.474 for peanut butter intake. Lines with dashes represent the 95% confidence intervals (CIs) for the fitted nonlinear trend (solid line).

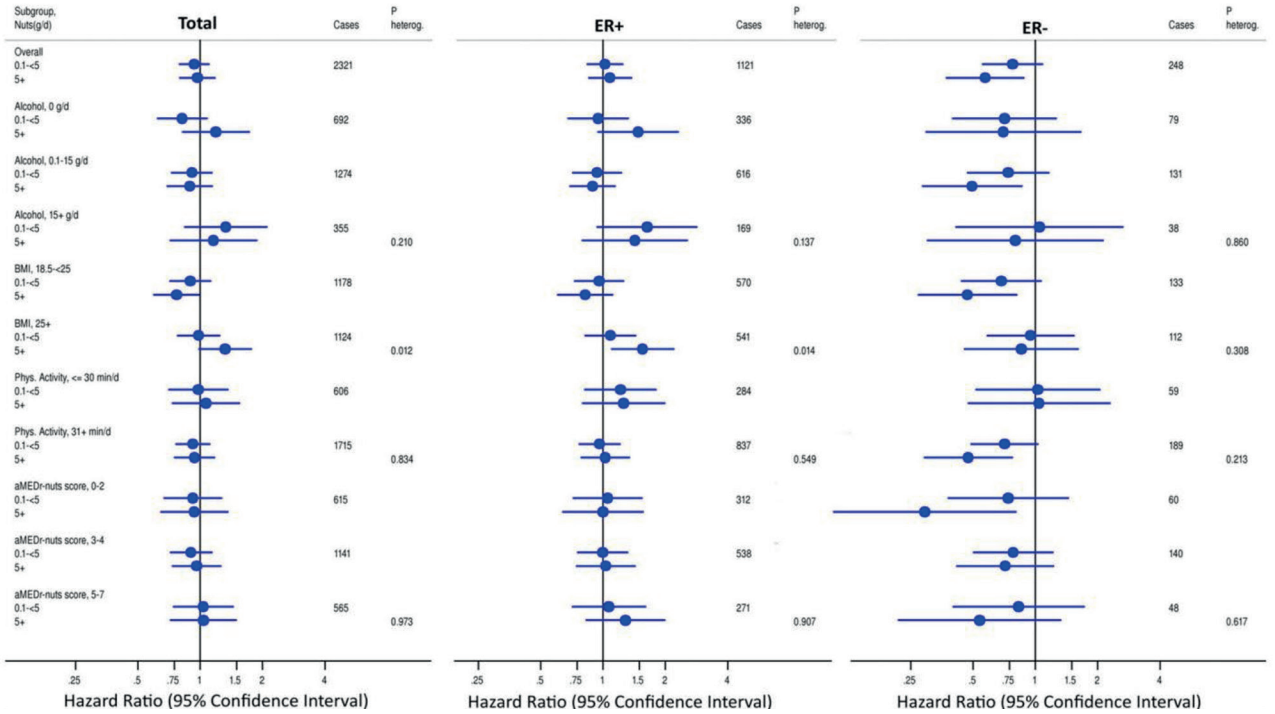


Figure 3. Hazard ratios and 95% confidence intervals of breast cancer, comparing total nut intake of 0.1-<5, and 5+ g/day versus 0 g/day, in subgroups of potential effect modifiers. Multivariable analyses were adjusted for the following: age at baseline (55-59, 60-64, 65-69 years), cigarette smoking (status (never, former, current), frequency (number of cigarettes per day; continuous, centered), duration (number of years; continuous, centered)), body height (continuous, cm), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), highest level of education (primary school or lower vocational, secondary or medium vocational, and higher vocational or university), family history of breast cancer in mother or sisters (no, yes), history of benign breast disease (no, yes), age at menarche (≤12, 13-14, 15-16, ≥17 years), parity (nulliparous, 1-2, ≥3 children), age at first birth (<25, ≥25 years), age at menopause (<45, 45-49, 50-54, ≥55 years), oral contraceptive use (never, ever), postmenopausal hormone replacement therapy (never, ever), energy intake (continuous, kcal/day), alcohol intake (0, 0.1-<5, 5-<15, 15-<30, ≥30 g/day), alternate Mediterranean Diet Score excluding alcohol and nuts (0-2, 3-4, 5-7 pts).

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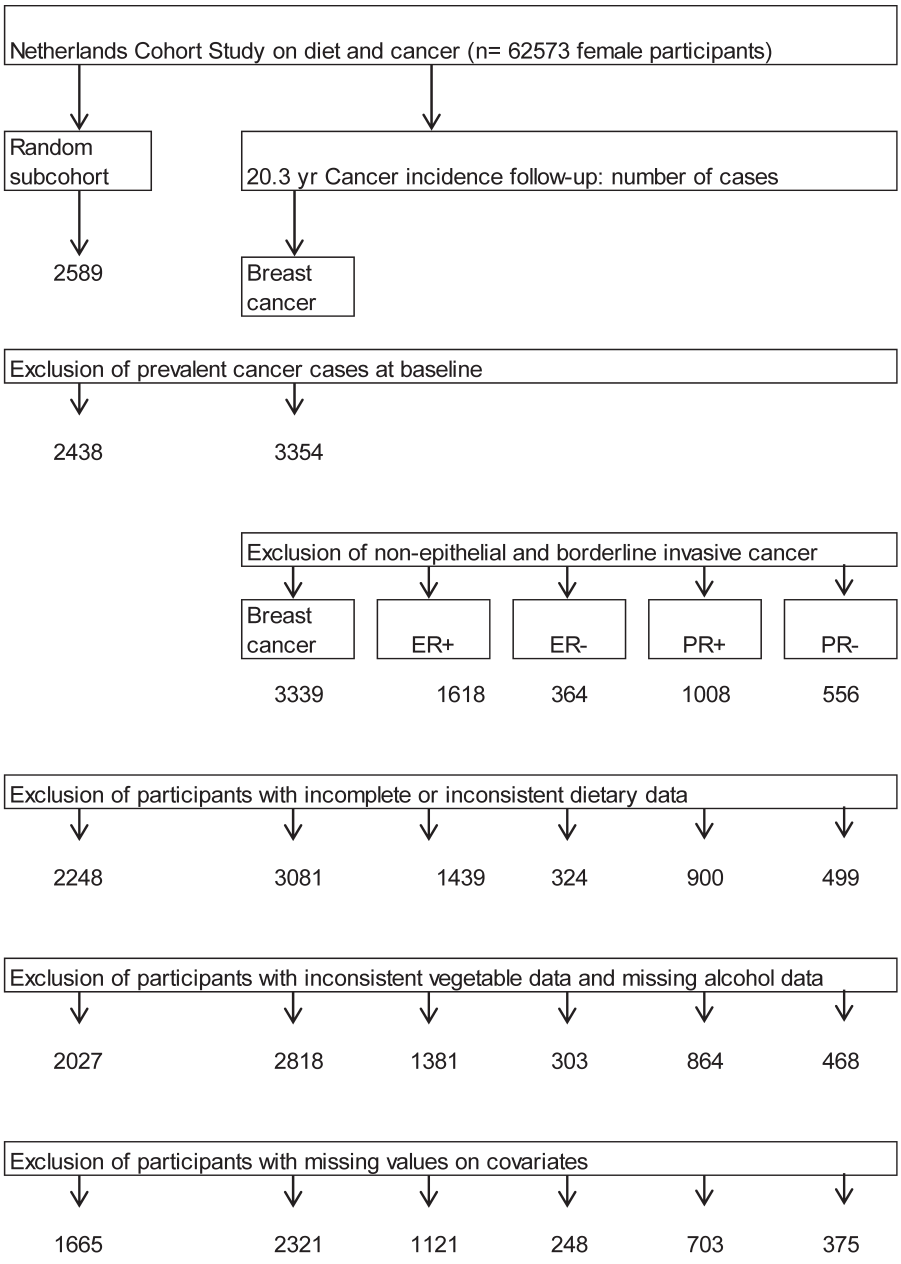

Supplementary Figure S1. Flow diagram of the number of subcohort members and cancer cases on which analyses are based, Netherlands Cohort Study.

Chapter 7

Nut and peanut butter intake are not directly associated with the risk of endometrial or ovarian cancer: Results from a Dutch prospective cohort study

Lisette Nieuwenhuis and Piet A. van den Brandt

Clin Nutr. 2020; 39: 2202-2210

Abstract

Background & aims: Nut intake has been associated with reduced cancer-related mortality and cancer risk. However, very few studies investigated the association between nut consumption and the risk of endometrial and ovarian cancer, with inconclusive results. We prospectively examined the relation between total nut, tree nut, peanut, and peanut butter intake and the risk of endometrial and ovarian cancer in the prospective Netherlands Cohort Study (NLCS).

Methods: In 1986, 62,573 women aged 55-69 years were included in the NLCS. At baseline, all participants filled in a questionnaire and a subcohort of 2589 women was randomly selected. After 20.3 years of follow-up, 389 endometrial and 347 ovarian cancer cases with complete data were included in the analysis. Hazard ratios (HRs) were calculated in multivariable-adjusted Cox regression analyses, using a case-cohort approach.

Results: Compared to nonconsumers, the HRs (95% confidence intervals) for women consuming 10+ g total nuts/day were 1.23 (0.82-1.87) for endometrial cancer and 0.84 (0.57-1.24) for ovarian cancer. For tree nut, peanut, and peanut butter intake, also no significant relations with endometrial or ovarian cancer were observed. In the endometrial cancer analyses, significant interactions of total nut intake with body mass index and cigarette smoking status were found.

Conclusions: The results of this study suggest that intake of total nuts, tree nuts, peanuts, and peanut butter is not related to the risk of endometrial or ovarian cancer. The observed interactions in the endometrial cancer analyses, in particular with cigarette smoking status, require confirmation in other studies.

Introduction

In 2012, uterine corpus cancer, which predominantly comprises endometrial cancer [1], was the fourth most common cancer in women in developed countries; ovarian cancer ranked fifth [2]. The development of endometrial cancer has mainly been linked to an excess of estrogen relative to progesterone [3]. For ovarian cancer, the most common explanation is the incessant ovulation hypothesis, which suggests that reproductive tissue turnover results in an accumulation of genetic damage [3-5]. Although endometrial and ovarian cancers are two distinct entities, these hypothesized mechanisms might apply to both cancer types [3]. Other proposed mechanisms for both cancer types relate, amongst others, to inflammation, gonadotropin stimulation, and mucin-related immunity [3, 5-7].

Recently, increased nut consumption has been associated with reduced cancer-related mortality and cancer risk [8-15]. Several animal and human studies stated that phytoestrogens in nuts (isoflavonoids and lignans) might modify sex hormone metabolism and activity, thereby possibly reducing the risk of hormone-dependent cancers [16, 17]. Other proposed mechanisms by which nuts have been suggested to conduct their cancer-chemopreventive effects relate, amongst others, to their antioxidant activity, regulation of immunological and anti-inflammatory responses, and regulation of cell proliferation and differentiation [16, 18-20].

Very few studies investigated the association between nut consumption and the risk of endometrial and ovarian cancer, with contradictive results: to our knowledge, only three case-control studies were performed for endometrial cancer [21-23], and one cohort [24] and two case-control studies for ovarian cancer [25, 26]. Because these studies are inconclusive and because prospective evidence regarding these relations is very limited, we investigated the role of tree nut, peanut, and peanut butter consumption in the development of endometrial and ovarian cancer in the prospective Netherlands Cohort Study on diet and cancer (NLCS).

Materials and methods

Study design and cancer follow-up

The NLCS was initiated in September 1986, when 62,573 women aged 55-69 years were enrolled [27]. These women agreed to participate by filling in and returning a baseline questionnaire, which measured dietary habits and other cancer risk factors. Ethical approval of the NLCS was obtained from the institutional review boards of the Maastricht University and the Netherlands Organization for Applied Scientific Research (TNO). The NLCS was conducted in accordance with the Declaration of Helsinki. A case-cohort approach was applied to improve the efficiency of the data processing and analysis. Following this approach, incident cases were derived from the entire cohort, whereas person-years at risk were estimated from a subcohort. This subcohort consisted of 2589 women who were randomly sampled from the total cohort directly after baseline. Subcohort members were

followed up biennially for vital status information until December 2006. After 20.3 years of follow-up (September 1986 until December 2006), no subcohort members were lost to follow-up.

Follow-up for cancer incidence was performed through annual record linkage with the Netherlands Cancer Registry and the Netherlands Pathology Registry (PALGA) [28]. The completeness of the cancer follow-up was estimated to be higher than 95% [29].

After 20.3 years of follow-up, 551 incident endometrial and 498 incident ovarian cancer cases were detected. Prevalent cancer cases (except for skin cancer), non-epithelial or borderline invasive cases, or cases without microscopic confirmation were excluded. Participants were excluded if they had a hysterectomy (excluded from the endometrial cancer analysis) or an oophorectomy (excluded from the ovarian cancer analysis). Moreover, cases and subcohort members with incomplete or inconsistent dietary data, or with missing data on confounders were also excluded. Applying these criteria resulted in 1452 subcohort members and 389 endometrial cancer cases for the analyses of endometrial cancer, and 1646 subcohort members and 347 ovarian cancer cases for the analyses of ovarian cancer (Figure 1).

Exposure assessment

Smoking habits, physical activity, anthropometrics, dietary intakes, and other cancer risk factors were evaluated with a mailed, self-administered, 11-page baseline questionnaire. Information about habitual diet in the year preceding baseline was assessed with a validated 150-item semi-quantitative food frequency questionnaire [30]. Intake of peanuts, tree nuts, and peanut butter was estimated by asking for intake frequencies and number of standard portion sizes consumed per intake of 'peanuts', 'other, mixed nuts' (tree nuts), and 'peanut butter'. Intake frequencies could range from 'never or less than 1x/month' to '6-7x/week'. A standard portion size was assumed 28 g for tree nuts and peanuts, and 15 g per slice of bread for peanut butter. Daily intakes were calculated by multiplying intake frequencies and portion sizes. Total nut intake was calculated as the sum of daily tree nut and peanut intake.

Statistical analysis

The relation between nut and peanut butter intake and the risk of endometrial and ovarian cancer was analyzed in age- and multivariable-adjusted Cox regression analyses. The proportional hazards (PH) assumption was evaluated with Schoenfeld residuals [31], log-log survival plots, and by including time-varying covariates. No violations of this assumption were observed in the endometrial and ovarian cancer analyses for the exposure variables. In case the PH assumption was violated for confounders, time-covariate interactions for those variables were included. Standard errors were calculated with the robust Huber-White sandwich estimator to account for the additional variance introduced by the sampling from the entire cohort [32].

The relation between nut and peanut butter intake and endometrial and ovarian cancer risk was tested on a categorical and continuous scale (per 5 g/day increment). For the categorical analyses, total nut and peanut intake were divided into categories of 0, 0.1-<5, 5-<10, and

10+ g/day, and tree nut and peanut butter intake into 0, 0.1-<5, and 5+ g/day, because of the lower number of cases in the higher intake categories. Linear trends were investigated by assigning median nut intake values in the subcohort to the intake categories and fitting these as a continuous variable in the regression models.

In the multivariable-adjusted models, estimates were adjusted for the following predefined confounders: age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), body mass index (BMI; <18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (primary or lower vocational (low), secondary or medium vocational (medium), higher vocational or university (high)), age at menarche (years; continuous), age at menopause (years; continuous), parity and age at first child birth (nulliparous, 1-2 children - <25 years, 1-2 children - ≥25 years, ≥3 children - <25 years, ≥3 children - ≥25 years), oral contraceptive use (never, ever), hormone replacement therapy use (never, ever), daily energy intake (kcal/day; continuous), and the alternate Mediterranean diet (aMED) score excluding alcohol and nuts [33] (0-2, 3-4, 5-7 points). In the endometrial cancer analyses, we additionally adjusted for family history of endometrial cancer (no, yes), and in the ovarian cancer analyses for family history of breast cancer (no, yes). Initially, we also adjusted the ovarian cancer analyses for family history of ovarian cancer. However, because only three participants reported a positive family history, this factor was excluded from the final model, which did not importantly change the estimates. We also checked the following potential confounders: intake of coffee, nutritional supplement use, history of diabetes (for the endometrial cancer analyses only), history of hypertension (for the endometrial cancer analyses only), hysterectomy (for the ovarian cancer analyses only), and height. Because these variables did not change the estimates with minimally 10% when using a backward stepwise selection procedure, they were excluded from the final model.

To further investigate the linearity of the exposure-response relation between nut and peanut butter intake and endometrial and ovarian cancer risk, we performed restricted cubic splines analyses with three fixed knots at 0, 5, and 10 g intake/day. To examine the assumptions regarding the number and placement of knots, we compared the fit of several models with additional knots or different knot positions using the Akaike Information Criterion (AIC) score [34].

Potential residual confounding and interactions were investigated by stratifying the relation between total nut intake and endometrial and ovarian cancer by BMI, nonoccupational physical activity, cigarette smoking status, educational level, and aMED score excluding alcohol and nuts. For ovarian cancer, we also investigated potential interactions by family history of breast cancer (no, yes). We could not stratify by family history of endometrial cancer (in the endometrial cancer analysis) or by family history of ovarian cancer (in the ovarian cancer analysis), because of the limited number of participants with a positive family history. The total nut intake categories of 5-<10 g/day and 10+ g/day were merged to increase statistical power. Participant with a BMI <18.5 kg/m^2 were excluded from the analysis stratified by BMI because of the small number of cases in this category. Interactions

were tested by including cross-product terms in the Cox models and performing Wald tests. To check for potential reversed causation, we excluded the first two years of follow-up. Secondly, we divided the total follow-up duration in two-year periods and compared the median baseline nut and peanut butter intake of cases diagnosed during these periods, using a Kruskal-Wallis test. Moreover, we restricted the analysis of peanut butter to participants who had stated having had a constant peanut butter intake in the five years preceding baseline. These data were not available for tree nut or peanut intake. In another sensitivity analyses, we adjusted for consumption of fruits, vegetables, dairy and cheese, and red and processed meat instead of the aMED score excluding alcohol and nuts. Furthermore, associations of tree nut, peanut, and peanut butter intake with endometrial and ovarian cancer were mutually adjusted.

Analyses were performed with Stata 15 software (StataCorp. 2017. College Station, TX). P-values were tested two-sided and were considered statistically significant if <0.05.

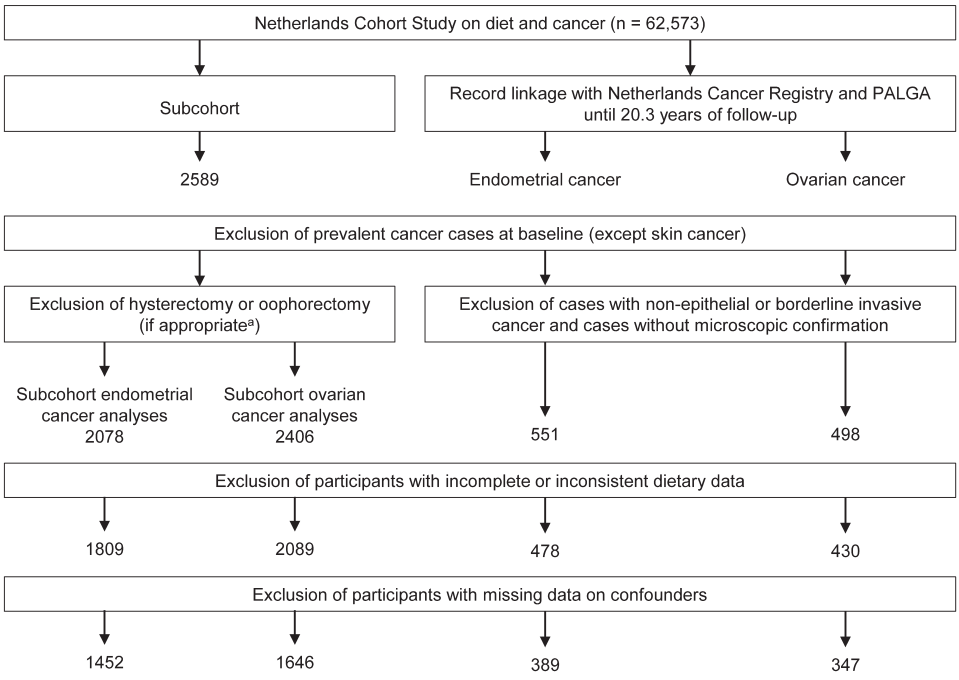

Figure 1. Flow chart of the number of subcohort members and ovarian and endometrial cancer cases; the NLCS, 1986-2006.
[a] Hysterectomy excluded from the analysis of endometrial cancer, oophorectomy excluded from the analysis of ovarian cancer.

Table 1. Baseline characteristics (mean (SD) or %) of subcohort members and endometrial and ovarian cancer cases in the Netherlands Cohort Study, 1986-2006

	Endometrial cancer		Ovarian cancer	
	Subcohort[a]	Cases	Subcohort[a]	Cases
N	1452	389	1646	347
Age (years)	61.4 (4.2)	61.4 (4.3)	61.3 (4.2)	61.5 (4.2)
Never cigarette smoker (%)	58.9	66.8	58.4	64.6
Body Mass Index (kg/m^2)	25.0 (3.5)	26.4 (4.1)	25.0 (3.5)	25.1 (3.6)
Nonoccupational physical activity (min/day)	66.3 (51.0)	58.6 (46.3)	66.0 (50.4)	57.8 (37.5)
University or higher vocational education (%)	9.7	8.7	9.7	9.5
Family history of endometrial cancer (%)	2.8	4.4		
Family history of ovarian cancer (%)			0.1	0.6
Family history of breast cancer (%)			8.7	7.8
Age at menarche (years)	13.7 (1.8)	13.4 (1.6)	13.7 (1.8)	13.7 (1.8)
Age at menopause (years)	49.1 (4.3)	50.2 (3.9)	48.9 (4.4)	49.3 (3.9)
Parous (%)	81.2	73.5	81.8	76.1
Age at first birth (in parous, years)	27.1 (4.2)	27.1 (3.9)	27.0 (4.2)	27.6 (4.1)
Number of children (in parous, n)	3.4 (1.9)	3.1 (1.7)	3.4 (1.9)	3.2 (1.7)
Ever used oral contraceptives (%)	24.5	13.9	25.3	19.0
Ever used hormone replacement therapy (%)	11.8	16.5	13.4	12.4
Daily energy intake (kcal)	1687 (390)	1658 (398)	1688 (392)	1695 (389)
Total nut intake (g/day)	4.2 (7.8)	4.4 (8.6)	4.4 (8.6)	4.2 (8.4)
Tree nut intake (g/day)	1.0 (2.7)	1.0 (3.0)	1.1 (4.1)	1.0 (2.9)
Peanut intake (g/day)	3.3 (6.8)	3.4 (6.9)	3.3 (6.9)	3.2 (6.6)
Peanut butter intake (g/day)	1.2 (3.6)	1.1 (3.2)	1.2 (3.5)	1.2 (3.7)
aMED score (excl. alcohol and nuts) of 5-7 pts (%)	26.5	23.1	26.6	23.9

[a] The subcohort sizes of the endometrial and ovarian cancer analyses differ because of differences in the in- and exclusion criteria (Figure 1)

Results

In the analyses of endometrial cancer, mean (SD) total nut intake was slightly higher in cases (4.4 (8.6) g/day) than in the subcohort (4.2 (7.8) g/day) (Table 1). In the ovarian cancer analyses, mean (SD) total nut intake was 4.2 (8.4) g/day among cases and 4.4 (8.6) g/day among subcohort members. Average intakes of tree nuts, peanuts, and peanut butter were almost similar in subcohort members and endometrial and ovarian cancer cases.

Regarding other baseline characteristics, both endometrial and ovarian cancer cases were on average less physically active and less often ever cigarette smokers, parous, or oral contraceptive users than subcohort members. Moreover, endometrial and ovarian cancer cases had a later mean age at menopause and scored lower on the aMED score excluding alcohol and nuts (Table 1). Furthermore, compared to subcohort members, endometrial cancer cases were on average heavier, lower educated, reported a positive family history of endometrial cancer more often, had a lower age at menarche, and used hormone replacement therapy more often. Ovarian cancer cases more often reported a positive family history of ovarian cancer than subcohort members, but less often a positive family history of breast cancer, and they used hormone replacement therapy less often.

Table 2. Age- and multivariable-adjusted HRs (and 95% CIs) for endometrial and ovarian cancer according to nut consumption; NLCS, 1986-2006

	Endometrial cancer					Ovarian cancer				
	Median intake[a]	Person-years	Cases	Age-adjusted HR (95% CI)	Multivariable-adjusted HR[b] (95% CI)	Median intake[a]	Person-years	Cases	Age-adjusted HR (95% CI)	Multivariable-adjusted HR[b] (95% CI)
Total nuts (g/day)										
0	0.0	9912	143	1.00 (reference)	1.00 (reference)	0.0	11,388	158	1.00 (reference)	1.00 (reference)
0.1-<5	2.1	9500	160	1.18 (0.91-1.53)	1.26 (0.94-1.67)	2.1	10,817	117	0.80 (0.61-1.04)	0.79 (0.59-1.05)
5-<10	7.8	2876	37	0.91 (0.60-1.38)	1.21 (0.76-1.92)	7.8	3115	28	0.68 (0.43-1.06)	0.71 (0.45-1.14)
10+	15.5	3338	49	1.03 (0.71-1.49)	1.23 (0.82-1.87)	15.7	3919	44	0.83 (0.57-1.20)	0.84 (0.57-1.24)
P_{trend}				0.743	0.449				0.305	0.425
Continuous, per 5 g/day increment				1.01 (0.93-1.08)	1.06 (0.97-1.14)				0.98 (0.91-1.06)	0.99 (0.91-1.07)
Tree nuts (g/day)										
0	0.0	17,973	277	1.00 (reference)	1.00 (reference)	0.0	20,505	248	1.00 (reference)	1.00 (reference)
0.1-<5	1.6	6204	93	0.98 (0.75-1.27)	1.03 (0.76-1.39)	1.6	7008	85	1.02 (0.78-1.33)	1.04 (0.77-1.41)
5+	8.9	1450	19	0.85 (0.51-1.43)	1.08 (0.62-1.90)	8.9	1727	14	0.69 (0.38-1.23)	0.71 (0.39-1.32)
P_{trend}				0.543	0.767				0.226	0.317
Continuous, per 5 g/day increment				0.99 (0.78-1.25)	1.06 (0.83-1.36)				0.94 (0.80-1.12)	0.96 (0.82-1.13)
Peanuts (g/day)										
0	0.0	11,772	175	1.00 (reference)	1.00 (reference)	0.0	13,535	182	1.00 (reference)	1.00 (reference)
0.1-<5	2.1	9548	151	1.08 (0.84-1.39)	1.20 (0.91-1.57)	2.0	10791	111	0.78 (0.60-1.02)	0.81 (0.61-1.06)
5-<10	8.5	2063	31	1.03 (0.66-1.61)	1.19 (0.73-1.96)	8.5	2249	21	0.73 (0.44-1.19)	0.75 (0.45-1.26)
10+	14.4	2242	32	0.97 (0.63-1.49)	1.16 (0.73-1.85)	17.1	2666	33	0.94 (0.63-1.43)	0.96 (0.62-1.47)
P_{trend}				0.896	0.499				0.699	0.792
Continuous, per 5 g/day increment				1.01 (0.93-1.09)	1.06 (0.98-1.16)				0.99 (0.90-1.08)	1.00 (0.91-1.10)
Peanut butter (g/day)										
0	0.0	18,388	298	1.00 (reference)	1.00 (reference)	0.0	21,173	257	1.00 (reference)	1.00 (reference)
0.1-<5	1.2	4654	58	0.77 (0.57-1.06)	0.83 (0.59-1.17)	1.2	5275	55	0.87 (0.63-1.20)	0.88 (0.63-1.21)
5+	5.3	2584	33	0.79 (0.53-1.18)	0.84 (0.54-1.30)	5.3	2792	35	1.05 (0.71-1.56)	1.02 (0.67-1.54)
P_{trend}				0.186	0.359				0.896	0.989
Continuous, per 5 g/day increment				0.92 (0.77-1.11)	0.96 (0.79-1.17)				1.02 (0.85-1.22)	1.00 (0.83-1.21)

[a] Median intake in the female subcohort.
[b] Adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of endometrial cancer (no, yes; in the endometrial cancer analysis only), family history of breast cancer (no, yes; in the ovarian cancer analysis only), age at menarche (years; continuous), age at menopause (years; continuous), parity and age at first child birth (nulliparous, 1-2 children - <25 years, 1-2 children - ≥25 years, ≥3 children - <25 years, ≥3 children - ≥25 years), oral contraceptive use (never, ever), hormone replacement therapy use (never, ever), daily energy intake (kcal/day; continuous), alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points).

Age- and multivariable-adjusted associations between nut and peanut butter intake and endometrial and ovarian cancer risk are presented in Table 2. In the age-adjusted analyses, no statistically significant relation of total nut intake was found with endometrial or ovarian cancer risk (HR (95% CI) for 10+ g/day vs. nonconsumers = 1.03 (0.71-1.49), p-trend = 0.743, and 0.83 (0.57-1.20), p-trend = 0.305, respectively). Tree nut, peanut, and peanut butter consumption were also not significantly related to endometrial or ovarian cancer risk in age-adjusted analyses. After multivariable-adjustment, the nonsignificant positive associations between total nut and peanut intake and endometrial cancer risk became somewhat stronger, whereas the nonsignificant inverse associations between tree nut and peanut butter intake and endometrial cancer risk attenuated or became positive. For ovarian cancer, multivariable-adjustment did not change the results importantly. Total nut intake was not significantly associated with endometrial or ovarian cancer risk after multivariable-adjustment (HR (95% CI) for 10+ g/day vs. nonconsumers = 1.23 (0.82-1.87), p-trend = 0.449, and 0.84 (0.57-1.24), p-trend = 0.452, respectively). Also no significant relations with endometrial or ovarian cancer were observed for tree nut, peanut, and peanut butter intake. In continuous analyses, nut and peanut butter consumption were also not related to the risk of endometrial or ovarian cancer.

In restricted cubic spline analyses with three fixed knots at 0, 5, and 10 g nut intake/day, no statistical evidence for nonlinear relations with endometrial or ovarian cancer risk were observed for all four exposure variables (Figure 2). However, the tests for nonlinearity were borderline significant for the relations between peanut butter intake and endometrial cancer risk (p-nonlinearity = 0.062) and between total nut intake and ovarian cancer risk (p-nonlinearity = 0.081). When using additional knots or different knot positions, the model fit, as measured with the AIC score, did not improve importantly (data not shown).

Table 3 and Supplementary Table 1 present the associations between total nut intake and endometrial and ovarian cancer risk in strata of potential effect modifiers. In the analyses of endometrial cancer stratified by BMI, no significant association between total nut intake and endometrial cancer risk was observed in participants with a BMI of 18.5-<25 kg/m^2 (Table 3). A nonsignificant positive trend was observed in participants with a BMI ≥25 kg/m^2, with a significantly increased risk in the category of 0.1-<5 g total nut intake/day compared to nonconsumers (HR (95% CI) = 1.68 (1.13-2.48)). The test for interaction by BMI was significant (p-interaction = 0.016). For cigarette smoking status, no relation between total nut intake and endometrial cancer risk was found in never smokers, a nonsignificant positive association in former smokers, and a significant positive trend in current smokers (HR (95% CI) for 5+ g/day vs nonconsumers = 3.49 (1.25-9.73), p-trend = 0.021). The p-interaction by smoking status was 0.019. In Figure 3, we further investigated the joint effects of total nut intake and cigarette smoking status on endometrial cancer risk, with never smokers who consumed 0 g total nuts/day as reference category. Increasing nut intake attenuated the inverse association between former cigarette smoking and endometrial cancer risk, and in women who consumed 5+ g total nuts/day, current smoking was even associated with a non-significantly increased endometrial cancer risk. In never smokers, no significant relation between nut intake and endometrial cancer was observed. Nevertheless, only currently

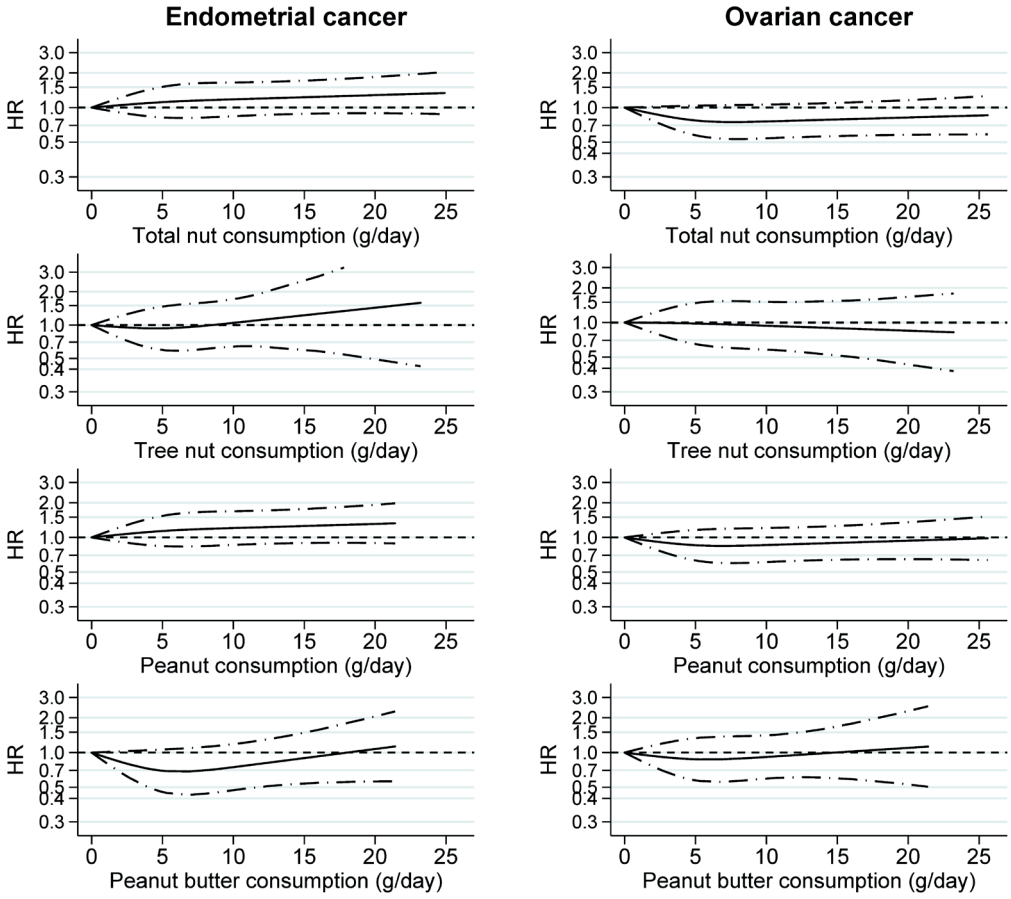

Figure 2. Restricted cubic spline analyses with three fixed knots at 0, 5, and 10 g intake/day, investigating the relation between nut and peanut butter consumption and the risk of endometrial and ovarian cancer. Solid lines represent HRs, dashed lines 95% confidence limits. P-values for nonlinearity for total nut, tree nut, peanut, and peanut butter intake were 0.724, 0.558, 0.640, and 0.062 for endometrial cancer, and 0.081, 0.911, 0.283, and 0.492 for ovarian cancer, respectively. Results were adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of endometrial cancer (no, yes; in the endometrial cancer analyses only), family history of breast cancer (no, yes; in the ovarian cancer analyses only), age at menarche (years; continuous), age at menopause (years; continuous), parity and age at first child birth (nulliparous, 1-2 children - <25 years, 1-2 children - ≥25 years, ≥3 children - <25 years, ≥3 children - ≥25 years), oral contraceptive use (never, ever), hormone replacement therapy use (never, ever), daily energy intake (kcal/day; continuous), alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points).

Table 3. Multivariable-adjusted associations between total nut intake and endometrial cancer risk in strata of potential effect modifiers; NLCS, 1986-2006

	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
Endometrial cancer					
Overall					
Cases/person-time at risk (years)	143/9912	160/9500	86/6214		
HR (95% CI)[a]	1.00 (reference)	1.26 (0.94-1.67)	1.22 (0.86-1.74)	0.410	
Body mass index[b]					
18.5-<25 kg/m^2					
Cases/person-time at risk (years)	65/4959	51/5085	48/4121		
HR (95% CI)[a]	1.00 (reference)	0.76 (0.49-1.18)	1.07 (0.65-1.77)	0.512	0.016
25+ kg/m^2					
Cases/person-time at risk (years)	76/4768	108/4343	38/2033		
HR (95% CI)[a]	1.00 (reference)	1.68 (1.13-2.48)	1.24 (0.73-2.09)	0.775	
Nonoccupational physical activity					
≤30 min/day					
Cases/person-time at risk (years)	46/2650	44/1752	23/1073		
HR (95% CI)[a]	1.00 (reference)	1.44 (0.83-2.50)	1.24 (0.56-2.73)	0.689	0.650
>30-≤60 min/day					
Cases/person-time at risk (years)	36/3029	56/3121	33/2109		
HR (95% CI)[a]	1.00 (reference)	1.74 (1.01-3.00)	1.61 (0.83-3.12)	0.341	
>60-≤90 min/day					
Cases/person-time at risk (years)	31/2153	32/2392	13/1447		
HR (95% CI)[a]	1.00 (reference)	0.93 (0.45-1.91)	0.84 (0.36-1.94)	0.682	
>90 min/day					
Cases/person-time at risk (years)	30/2080	28/2235	17/1585		
HR (95% CI)[a]	1.00 (reference)	0.97 (0.40-2.33)	0.96 (0.36-2.54)	0.937	
Cigarette smoking status					
Never					
Cases/person-time at risk (years)	106/6166	114/5791	40/3320		
HR (95% CI)[a]	1.00 (reference)	1.21 (0.86-1.71)	0.83 (0.51-1.35)	0.322	0.019
Former					
Cases/person-time at risk (years)	20/1507	28/2148	24/1758		
HR (95% CI)[a]	1.00 (reference)	1.23 (0.55-2.78)	1.32 (0.56-3.07)	0.594	
Current					
Cases/person-time at risk (years)	17/2239	18/1562	22/1137		
HR (95% CI)[a]	1.00 (reference)	1.97 (0.78-4.94)	3.49 (1.25-9.73)	0.021	
Educational level					
Low					
Cases/person-time at risk (years)	84/6013	92/4941	37/2673		
HR (95% CI)[a]	1.00 (reference)	1.50 (1.02-2.21)	1.32 (0.79-2.21)	0.397	0.620
Medium					
Cases/person-time at risk (years)	49/3316	54/3507	39/2696		
HR (95% CI)[a]	1.00 (reference)	1.05 (0.62-1.77)	1.11 (0.59-2.09)	0.752	
High					
Cases/person-time at risk (years)	10/583	14/1052	10/845		
HR (95% CI)[a]	1.00 (reference)	0.84 (0.14-5.16)	0.64 (0.10-4.05)	0.576	

(Continued)	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
Adapted Mediterranean diet score excluding nuts and alcohol					
0-2 points					
Cases/person-time at risk (years)	35/2980	50/2088	17/1372		
HR (95% CI)[a]	1.00 (reference)	2.22 (1.15-4.28)	1.27 (0.54-2.99)	0.883	0.169
3-4 points					
Cases/person-time at risk (years)	79/4855	74/4596	44/2875		
HR (95% CI)[a]	1.00 (reference)	1.04 (0.69-1.57)	1.23 (0.74-2.05)	0.424	
5-7 points					
Cases/person-time at risk (years)	29/2077	36/2817	25/1967		
HR (95% CI)[a]	1.00 (reference)	0.87 (0.43-1.74)	1.05 (0.48-2.28)	0.720	

[a] Adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of endometrial cancer (no, yes), age at menarche (years; continuous), age at menopause (years; continuous), parity and age at first child birth (nulliparous, 1-2 children - <25 years, 1-2 children - ≥25 years, ≥3 children - <25 years, ≥3 children - ≥25 years), oral contraceptive use (never, ever), hormone replacement therapy use (never, ever), daily energy intake (kcal/day; continuous), alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points).

[b] Participants with a BMI <18.5 kg/m^2 (n = 22) were excluded from the interaction analysis.

smoking nonconsumers had a significantly lower endometrial cancer risk than never smoking nonconsumers (HR (95% CI) = 0.45 (0.25-0.81)). For ovarian cancer, no significant interactions between total nut intake and potential effect modifiers were observed (Supplementary Table 1).

No significant differences were found in the median baseline nut and peanut butter intake of endometrial and ovarian cancer cases diagnosed over the follow-up period in Kruskal-Wallis tests (p ≥0.206) (data not shown). Exclusion of the first two years of follow-up resulted in similar results as when the total follow-up period was included (data not shown). Moreover, restricting the analyses of the relation between peanut butter intake and endometrial and ovarian cancer risk to those participants who had stated having had a constant peanut butter intake in the five years before baseline also did not importantly change the results (data not shown).

In another sensitivity analysis, adjustment for intake of fruits, vegetables, dairy and cheese, and red and processed meat gave similar estimates as when adjusting for the aMED score excluding nuts and alcohol (data not shown). Moreover, mutually adjusting intake of tree nuts, peanuts, and peanut butter in relation to endometrial and ovarian cancer risk also did not change the results (data not shown).

Discussion

In the current study, total nut intake was not significantly related to the risk of endometrial or ovarian cancer. Similar results were found for tree nut, peanut, and peanut butter intake.

For the relation between total nuts and endometrial cancer risk, we observed significant interactions by BMI and cigarette smoking status.

Our results for ovarian cancer are in line with the results from the Swedish Women's Lifestyle and Health Cohort Study [24], in which also no statistically significant association between nut consumption and ovarian cancer risk was observed. To our knowledge, this is the only other prospective cohort study investigating the relation between nut intake and ovarian cancer risk. No other prospective evidence is available for endometrial cancer.

Besides the abovementioned cohort study, only two case-control studies have been performed on this topic for ovarian cancer [25, 26], and three case-control studies for endometrial cancer [21-23]. Regarding ovarian cancer, a Canadian case-control study did not find a relation between nut product intake frequency and ovarian cancer risk [26], and

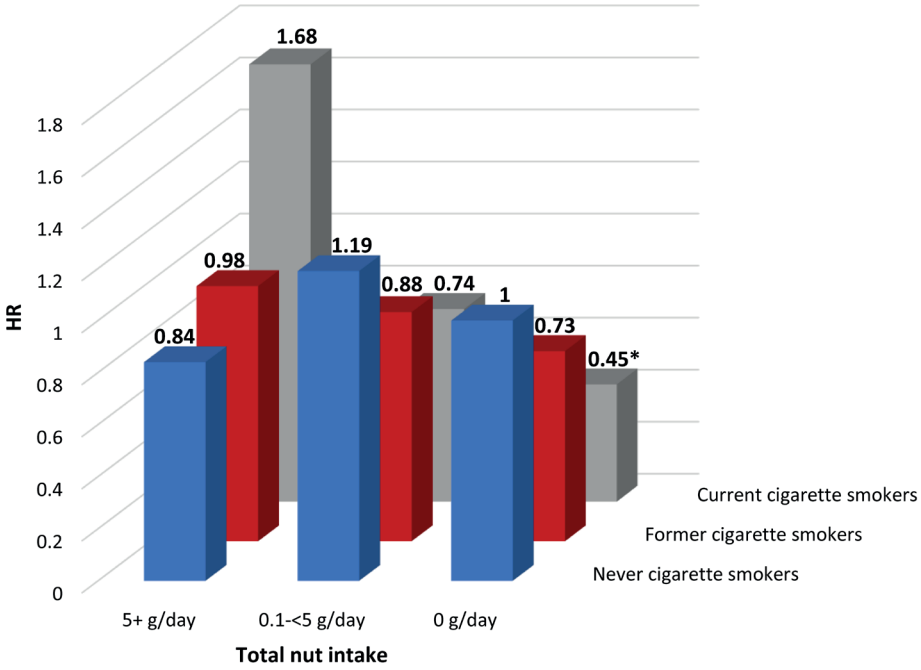

Figure 3. Combined exposure to total nuts and cigarette smoking and the risk of endometrial cancer; the NLCS, 1986-2006. Never cigarette smokers who consumed 0 g nuts/day are the reference category. Results were adjusted for age (years; continuous), cigarette smoking (frequency (n/day; continuous, centered) and duration (years; continuous, centered)), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2); nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of endometrial cancer (no, yes), age at menarche (years; continuous), age at menopause (years; continuous), parity and age at first child birth (nulliparous, 1-2 children - <25 years, 1-2 children - ≥25 years, ≥3 children - <25 years, ≥3 children - ≥25 years), oral contraceptive use (never, ever), hormone replacement therapy use (never, ever), daily energy intake (kcal/day; continuous), alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points)
* indicates a significant association ($p<0.05$)

in an Australian case-control study, intake of omega-6 fatty acids from nuts was significantly associated with a reduced risk of epithelial ovarian cancer [25]. Because the relation of omega-6 fatty acids with ovarian cancer risk varied between the food sources of the omega-6 fatty acids, the authors stated that the estimates probably reflect a relation with nuts rather than with omega-6 fatty acids [25].

Regarding endometrial cancer, one case-control study in Greece observed significant positive associations for intake of pulses and nuts combined [23], whereas a later Greek case-control study found a significant inverse association for pulse, nut, and seed consumption together [21]. In a Japanese case-control study, consuming peanuts ≥1-2x/week was associated with a significantly reduced risk of endometrial endometrioid carcinoma [22]. A borderline significant inverse trend was seen when peanut intake was expressed as intake density (g/1000 kcal) [22]. Case-control studies are prone to selection and information biases, which may explain the contradictive results for both endometrial and ovarian cancer. Furthermore, none of the above-mentioned studies investigated the interaction between nut intake and cigarette smoking. Thus, the evidence on the relation between nut intake and endometrial and ovarian cancer is very limited, and further (prospective) research is required to confirm our results.

For endometrial cancer, we observed significant interactions of total nut intake with BMI. However, only the category of 0.1-<5 g total nut intake/day was significantly associated with an increased endometrial cancer risk in participants with a BMI higher than 25 kg/m^2, and no significant exposure-response trends were observed in both BMI strata. Because of the number of significance tests performed, this finding may be due to chance. Nuts are energy-dense foods, and therefore concerns have been raised about weight gain resulting from increased nut intake. In case of hormone-dependent cancers, like endometrial and ovarian cancer, this is especially important because of the hormonal activity of adipose tissue [3, 35, 36]. However, several cross-sectional and prospective studies have indicated that higher nut intake is actually associated with reduced weight gain and a lower risk of becoming overweight or obese [37-40].

The interaction between total nut intake and cigarette smoking in relation to endometrial cancer risk was also significant. In contrast to most cancer sites, cigarette smoking has been associated with a lower risk of endometrial cancer, particularly among postmenopausal women [41, 42]. This protective effect is hypothesized to be related to a reduction in the level of circulating unopposed estrogens: smoking has been found to modify the production and metabolism of estrogens, androgens, and progesterone, and to reduce body weight [41-43]. Moreover, smoking might have direct cytotoxic effects on the ovaries, which causes oocyte destruction and induces earlier menopause [42, 43]. In our study, increasing total nut intake appeared to attenuate the protective effect of smoking (Figure 3), and even a non-significantly increased endometrial cancer risk was found in current smokers who consumed at least 5 g nuts/day. One possible explanation for this observation is that phytoestrogens in nuts might have estrogenic activity if the circulating concentration of unopposed endogenous estrogens is low [17, 44], which possibly counteracts the protective

antiestrogenic effects of smoking. Nuts also contain several components with antioxidant, anti-inflammatory, and cell metabolism-modifying properties [16, 19], which might also potentially oppose the effects of smoking. However, this is the first study investigating the interaction between nut intake and cigarette smoking in relation to endometrial cancer risk, and this finding needs to be confirmed in other studies first.

Our study has some limitations. Only baseline measurements were performed, while dietary intakes may have changed over the 20.3 year follow-up period. Nevertheless, dietary habits appeared to be quite stable for at least five years in a reproducibility study [45]. Potential measurement error might have resulted in misclassification and thus in an attenuation of the results. Moreover, potential residual confounding by measured and unmeasured confounders cannot be excluded. For example, we had no information on risk factors like breastfeeding and tubal ligation. Because these factors are unlikely to be associated with nut intake, they are not expected to confound our results.

Strengths of the study are the prospective nature and the long and complete follow-up, which make selection and information bias unlikely. The large number of participants allowed us to extensively correct for potential confounders. Moreover, we were able to distinguish between tree nut, peanut, and peanut butter intake.

In conclusion, the results of this prospective cohort study suggest that total nut, tree nut, peanut, and peanut butter intake are not related to the risk of endometrial or ovarian cancer. The observed interactions of nut intake in relation to endometrial cancer risk, in particular with cigarette smoking, need confirmation in other studies.

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Supplementary Table 1. Multivariable-adjusted associations between total nut intake and ovarian cancer risk in strata of potential effect modifiers; NLCS, 1986-2006

	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
Ovarian cancer					
Overall					
Cases/person-time at risk (years)	158/11,388	117/10,817	72/7034		
HR (95% CI)[a]	1.00 (reference)	0.79 (0.60-1.05)	0.78 (0.56-1.10)	0.258	
Body mass index[b]					
18.5-<25 kg/m^2					
Cases/person-time at risk (years)	78/5765	65/5651	38/4666		
HR (95% CI)[a]	1.00 (reference)	0.96 (0.65-1.42)	0.71 (0.45-1.13)	0.135	0.194
25+ kg/m^2					
Cases/person-time at risk (years)	80/5389	51/5095	32/2309		
HR (95% CI)[a]	1.00 (reference)	0.61 (0.39-0.95)	0.85 (0.50-1.46)	0.869	
Nonoccupational physical activity					
≤30 min/day					
Cases/person-time at risk (years)	44/2942	24/2033	17/1287		
HR (95% CI)[a]	1.00 (reference)	0.73 (0.38-1.38)	0.77 (0.37-1.57)	0.534	0.718
>30-≤60 min/day					
Cases/person-time at risk (years)	56/3411	46/3580	21/2418		
HR (95% CI)[a]	1.00 (reference)	0.77 (0.47-1.26)	0.59 (0.32-1.10)	0.125	
>60-≤90 min/day					
Cases/person-time at risk (years)	34/2633	27/2720	22/1657		
HR (95% CI)[a]	1.00 (reference)	0.69 (0.36-1.32)	0.95 (0.47-1.93)	0.827	
>90 min/day					
Cases/person-time at risk (years)	24/2403	20/2485	12/1672		
HR (95% CI)[a]	1.00 (reference)	0.89 (0.42-1.91)	0.85 (0.35-2.06)	0.743	
Cigarette smoking status					
Never					
Cases/person-time at risk (years)	103/7077	82/6612	39/3677		
HR (95% CI)[a]	1.00 (reference)	0.84 (0.59-1.19)	0.73 (0.47-1.13)	0.193	0.399
Former					
Cases/person-time at risk (years)	27/1744	19/2444	17/2008		
HR (95% CI)[a]	1.00 (reference)	0.47 (0.22-0.99)	0.48 (0.21-1.08)	0.219	
Current					
Cases/person-time at risk (years)	28/2567	16/1761	16/1349		
HR (95% CI)[a]	1.00 (reference)	0.94 (0.44-1.99)	1.53 (0.66-3.51)	0.283	
Educational level					
Low					
Cases/person-time at risk (years)	87/6909	60/5730	41/3156		
HR (95% CI)[a]	1.00 (reference)	0.85 (0.58-1.26)	1.08 (0.67-1.73)	0.654	0.304
Medium					
Cases/person-time at risk (years)	60/3780	43/3904	23/2933		
HR (95% CI)[a]	1.00 (reference)	0.62 (0.38-0.99)	0.47 (0.26-0.85)	0.031	
High					
Cases/person-time at risk (years)	11/669	14/1183	8/946		
HR (95% CI)[a]	1.00 (reference)	2.38 (0.39-14.46)	0.92 (0.18-4.57)	0.583	

(Continued)	Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
	0 g/day	0.1-<5 g/day	5+ g/day		
Family history of breast cancer					
No					
Cases/person-time at risk (years)	147/10,472	107/9679	66/6527		
HR (95% CI)[a]	1.00 (reference)	0.81 (0.60-1.10)	0.79 (0.56-1.12)	0.267	0.699
Yes					
Cases/person-time at risk (years)	11/916	10/1138	6/507		
HR (95% CI)[a]	1.00 (reference)	0.53 (0.13-2.18)	0.83 (0.18-3.75)	0.960	
Adapted Mediterranean diet score excluding nuts and alcohol					
0-2 points					
Cases/person-time at risk (years)	41/3362	37/2430	15/1506		
HR (95% CI)[a]	1.00 (reference)	1.36 (0.71-2.61)	0.75 (0.36-1.55)	0.299	0.165
3-4 points					
Cases/person-time at risk (years)	82/5625	56/5199	33/3306		
HR (95% CI)[a]	1.00 (reference)	0.72 (0.48-1.08)	0.72 (0.44-1.18)	0.286	
5-7 points					
Cases/person-time at risk (years)	35/2401	24/3188	24/2223		
HR (95% CI)[a]	1.00 (reference)	0.55 (0.29-1.04)	0.98 (0.47-2.01)	0.620	

[a] Adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), educational level (low, medium, high), family history of breast cancer (no, yes), age at menarche (years; continuous), age at menopause (years; continuous), parity and age at first child birth (nulliparous, 1-2 children - <25 years, 1-2 children - ≥25 years, ≥3 children - <25 years, ≥3 children - ≥25 years), oral contraceptive use (never, ever), hormone replacement therapy use (never, ever), daily energy intake (kcal/day; continuous), alternate Mediterranean diet score excluding alcohol and nuts (0-2, 3-4, 5-7 points).

[b] Participants with a BMI <18.5 kg/m^2 (n = 25) were excluded from the interaction analysis.

Chapter 8

Total nut, tree nut, peanut, and peanut butter intake and the risk of prostate cancer in the Netherlands Cohort Study

Esther A. Boudewijns, Lisette Nieuwenhuis, Milan S. Geybels, and Piet A. van den Brandt

Prostate Cancer Prostatic Dis. 2019; 22: 467-474

Abstract

Background: The consumption of nuts has been associated with a reduction of cancer risk, but only a few studies have examined the effects of nuts on prostate cancer risk. The current study prospectively investigated the association between the consumption of total nuts, tree nuts, peanuts, and peanut butter and the risk of total, advanced, and non-advanced prostate cancer.

Methods: The association between nuts and prostate cancer was evaluated in the Netherlands Cohort Study, which was conducted among 58,279 men aged 55-69 year at baseline. A case-cohort approach was used for data processing and analyses. After 20.3 years of follow-up, 3868 incident prostate cancer cases and 1979 subcohort members were available for multivariable Cox regression analyses.

Results: For total, advanced, and non-advanced prostate cancer, no significant associations were found for total nuts (total prostate cancer: hazard ratio (HR) (95%CI) for 10+ g/day vs. nonconsumers = 1.09 (0.92-1.29), P_{trend} = 0.409). No significant associations were observed for tree nuts and peanuts for total, advanced, and non-advanced prostate cancer risk. Peanut butter consumption was associated with a significantly increased risk of non-advanced prostate cancer (HR (95%CI) for 5+ g/day vs. nonconsumers = 1.33 (1.08-1.63), P_{trend} = 0.008), but not with total or advanced prostate cancer.

Conclusions: No significant associations were found between total nut, tree nut, and peanut consumption and total, advanced, and non-advanced prostate cancer. Peanut butter might be associated with an increased non-advanced prostate cancer risk.

Introduction

Prostate cancer is the second most common cancer and the fifth leading cause of cancer death in men worldwide[1]. Several studies have investigated the association between etiological factors and prostate cancer risk, but the evidence has not been consistent[2]. This might be due to the heterogeneous nature of prostate cancer. Studies have indicated that risk factors for advanced and non-advanced prostate cancer differ, which can most likely be explained by the way they act on biological pathways[3]. Advanced prostate cancer cases have a higher diagnostic certainty, and often have stronger associations with etiological factors than non-advanced prostate cancer cases. Non-advanced prostate cancer cases can therefore dilute the association towards the null if only total prostate cancer cases are investigated[2,3]. Three risk factors for prostate cancer have been well-established: age, ethnicity, and a positive family history of prostate cancer[3,4]. Potential risk factors for prostate cancer for which moderate to strong evidence from meta-analyses is available include body mass index (BMI) and height, and potential risk factors for which limited evidence is available include diabetes mellitus type 2, alcohol consumption, smoking, physical activity, and diet[5-14], whereby the associations are often more pronounced for advanced than for non-advanced prostate cancer.

One food group that has a potential to reduce prostate cancer risk are nuts. Nut intake has consistently been associated with a reduction of several chronic conditions, including cancer risk and cancer-related mortality[15-17]. Although the exact mechanism is unclear, it is suggested that their beneficial impact relates to their antioxidant and anti-inflammatory properties[18,19].

Only two prospective studies[20,21] and three case-control studies[22-24] have examined the association between the consumption of nuts and prostate cancer incidence or mortality. They show inconsistent results, demonstrating either a protective or a nonsignificant association. Moreover, studies that investigated the different effects on advanced and non-advanced prostate cancer risk are limited. The aim of the current study is to prospectively investigate the association between the consumption of total nuts, tree nuts, peanuts, and peanut butter, and the risk of total, advanced, and non-advanced prostate cancer.

Methods

Study design and population

The current study was performed within the Netherlands Cohort Study (NLCS). The study started on 17 September 1986 and included 58,279 men. A detailed description of the study is reported elsewhere[25]. For efficiency reasons, a case-cohort design was used, with cases derived from the entire cohort and the person-years at risk estimated in a subcohort. At baseline, participants consented to participate by completing and returning a mailed self-administered questionnaire, including a 150-item semi-quantitative food frequency questionnaire (FFQ). A subcohort of 2411 men was randomly selected from the full cohort

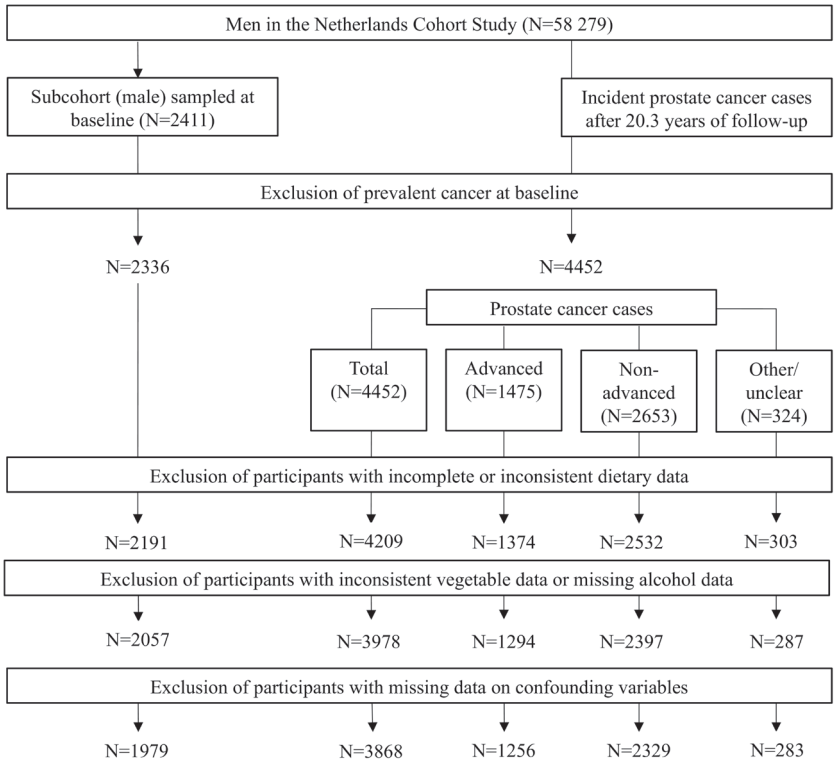

Figure 1. Flow diagram of included cases and subcohort member

at the time of baseline measurement. Data on vital status was collected in the subcohort during the follow-up period of 20.3 years (until 31 December 2006), and was 100% complete. Ethical clearance was obtained from the University Hospital Maastricht (Maastricht, the Netherlands) and the Netherlands Organisation for applied scientific research (TNO).

Data on incident prostate cancer cases in the entire cohort were collected using record linkage with the Netherlands Cancer Registry and the Dutch National Pathology Registry (PALGA)[26]. All cases were microscopically verified. The completeness of the cancer incidence follow-up was estimated to be at least 96%[27]. Prostate cancer cases were classified using the guidelines of the International Union Against Cancer, with non-advanced prostate cancer cases classified as stage T1 or T2, and N0 and M0, and advanced prostate cancer cases classified as stage T3 or T4, N+, or M1.

A flow diagram of the number of subcohort members and cases in the current study can be found in Figure 1. Cases and subcohort members with prevalent cancer (except for skin cancer) at baseline, with incomplete or inconsistent dietary data, or missing data on confounding variables were excluded from the analysis[28]. The study population in the current study includes 1979 subcohort members and 3868 incident prostate cancer cases diagnosed

during the 20.3 years follow-up. Of these cases, 2329 were classified as non-advanced prostate cancer cases and 1256 as advanced prostate cancer cases. The classification for 283 cases was unclear.

Exposure measurement

The baseline questionnaire measured the potential risk factors for cancer, including anthropometry, smoking habits, physical activity, and disease history. A validated FFQ assessed habitual diet in the preceding year[28]. The frequency of consumption of 'peanuts', 'other nuts, mixed nuts' (tree nuts), and 'peanut butter' was measured based on the following range: 'never or less than 1 time/month' to '6-7 times per week'. In addition, the number of standard portion sizes per intake was reported by participants. The standard portion size was 28 grams for tree nuts and peanuts, and 15 grams per slice of bread for peanut butter. The mean daily intake of nuts was calculated in grams, by multiplying the frequency of consumption by portion sizes. Total nut consumption consisted of peanut and tree nut intake. The personnel that were involved in entry, coding, and interpretation of the questionnaire were blinded to the case/subcohort status of the participants.

Statistical analysis

Cox proportional hazard models were used to estimate hazard ratios (HRs) and 95% CIs. The consumption of total nuts, tree nuts, peanuts, and peanut butter were analysed separately, both on a categorical and continuous scale. Categories were divided as follows: 0, 0.1-<5, 5-<10, and 10+ g/day for total nuts and peanuts, and 0, 0.1-<5, and 5+ g/day for tree nuts and peanut butter. The intake category of 0 g/day was used as reference group. The median value of nut consumption per category in the subcohort was used as a continuous variable in a Cox regression model to assess linear trends. For the continuous analyses, results were expressed in HRs per 5 g/day increment. All analyses were performed for total, non-advanced, and advanced prostate cancer, and Wald tests were performed to evaluate the statistical significance.

To control for the additional variance introduced by using the person-years at risk from the subcohort, a Huber-White sandwich estimator was used to calculate standard errors[29]. The proportional hazards assumption was tested with the scaled Schoenfeld residuals, -log-log survival plots, and time-covariate interaction terms. No violation of this assumption was observed for the exposure variables. Time-varying covariates were included in the model if the assumption was violated for confounders.

Factors that are known or hypothesized to be associated with prostate cancer and nut intake based on literature were assessed as confounders. Predefined confounders were included in the final model irrespective of their effect on the estimates, and include: age (years; continuous), family history of prostate cancer (yes/no), alcohol consumption (g/day; continuous), level of education (primary school or lower vocational education (low)/secondary school or medium vocational education (medium)/university or higher vocational education (high)), BMI (<18.5/18.5-<25/25-<30/≥30 kg/m^2), and total energy intake (kcal/day; continuous). Potential confounders include: height (cm; continuous), nonoccupational

physical activity (≤30/>30-≤60/>60-≤90/>90 min/day), cigarette smoking status (never/former/current), cigarette smoking frequency (n/day; continuous centred), cigarette smoking duration (years; continuous centred), history of diabetes (yes/no), nutritional supplement use (yes/no), tea consumption (<1 time per week/1-<2 times per week/2-<4 times per week/4-<5 times per week/≥5 times per week), intake of fruit (g/day; continuous), and intake of vegetables (g/day; continuous). Potential confounders were included if the variable changed the HR by at least 10% when using a backward stepwise selection procedure. In the final multivariable-adjusted model, only the predefined confounders were included.

For the interaction analysis, the following predefined strata were considered: family history of prostate cancer (yes/no), baseline BMI (18.5-<25/≥25 kg/m^2), alcohol consumption (0/0.1-<15/≥15 g/day), and educational level (low/medium/high). To test for interactions, cross-product terms were included in the Cox regression models, and Wald tests were performed. Participants with a BMI <18.5 kg/m^2 were deleted from the interaction analysis to increase statistical power.

The analysis was repeated with the first two years of follow-up excluded to assess whether preclinical disease influenced the observed associations. Besides, all nut and peanut butter consumers were excluded from the reference group to assess whether this would substantially change the results. Lastly, the analyses were repeated with mutual adjustment for the effects of other nut groups.

Statistical analysis was performed using Stata software (Version 15.0; Stata Corporation, College Station, TX), and differences were considered statistically significant at P <0.05 for two-sided testing.

Results

The mean consumption (SD) of total nuts, tree nuts, peanuts, and peanut butter in the male subcohort was 7.8 (13.8), 1.0 (3.3), 6.8 (13.1), and 1.4 (4.1) g/day, respectively. Baseline characteristics are presented in Table 1. As compared to the subcohort, total prostate cancer cases more often had a family history of prostate cancer, less often had a history of diabetes, and they were higher educated.

Table 2 presents the age- and multivariable-adjusted HRs for total, advanced, and non-advanced prostate cancer according to total nut, tree nut, peanut, and peanut butter consumption. In the age-adjusted model, no significant associations were observed for the highest versus the lowest intake category of total nuts, tree nuts, and peanuts in total, advanced, and non-advanced prostate cancer cases. Peanut butter intake was associated with a statistically significant increased risk of non-advanced prostate cancer, but not with total or advanced prostate cancer. The addition of covariates to the multivariable-adjusted model slightly attenuated the HRs as compared to the age-adjusted model. In the

Table 1. Baseline characteristics (mean (SD) or percentage) of subcohort members and prostate cancer cases from the Netherlands Cohort Study, 1986-2006

	Subcohort	Prostate cancer		
		Total	Advanced	Non-advanced
N*	1979	3868	1256	2329
Age (years)	61.3 (4.2)	61.8 (4.1)	61.7 (4.1)	61.6 (4.1)
Height (cm)	176.5 (6.6)	176.6 (6.7)	176.5 (6.4)	176.8 (6.8)
BMI (kg/m^2)	24.9 (2.6)	25.0 (2.5)	25.0 (2.5)	24.9 (2.5)
Nonoccupational physical activity (min/day)	80.8 (67.7)	79.9 (61.9)	77.9 (59.9)	80.7 (62.9)
Ever smokers (%)	87.2	85.5	85.9	85.1
Higher vocational school or university (%)	19.7	23.0	20.8	24.6
Family history of prostate cancer (%)	2.4	3.5	3.3	3.7
History of diabetes (%)	3.2	2.7	2.8	2.7
Dietary intake				
Intake of total nuts (g/day)	7.8 (13.8)	8.3 (15.2)	8.5 (16.5)	8.3 (14.6)
Intake of tree nuts (g/day)	1.0 (3.3)	1.1 (3.8)	1.1 (3.9)	1.1 (3.7)
Intake of peanuts (g/day)	6.8 (13.1)	7.1 (14.1)	7.4 (15.6)	7.1 (13.3)
Intake of peanut butter (g/day)	1.4 (4.1)	1.6 (4.4)	1.5 (4.2)	1.8 (4.7)
Total energy intake (kcal/day)	2166 (498)	2159 (487)	2169 (493)	2164 (482)
Alcohol consumption (g/day)	15.1 (17.0)	14.9 (15.6)	14.8 (15.3)	14.9 (15.6)
Tea consumption ≥2 cups/day (%)	68.5	69.4	67.2	70.2
Intake of fruit (g/day)	154.8 (114.4)	161.5 (111.8)	157.1 (109.7)	164.8 (112.6)
Intake of vegetables (g/day)	186.5 (75.6)	188.8 (73.4)	190.1 (75.7)	188.7 (72.3)
Nutritional supplement user (%)	23.2	23.9	22.8	24.3

*Excluding participants with prevalent cancer (except for skin cancer) at baseline, incomplete or inconsistent dietary data, or missing data on confounding variables (age, family history of prostate cancer, level of education, energy intake, alcohol consumption, and body mass index)

multivariable-adjusted analysis of total prostate cancer, no significant association was found for total nuts (HR (95%CI) for 10+ g/day vs. nonconsumers = 1.09 (0.92-1.29), P_{trend} = 0.409). Also, no significant associations were found for tree nuts, peanuts, or peanut butter in total prostate cancer cases. In advanced prostate cancer cases, no significant association was found for total nuts (HR (95%CI) for 10+ g/day vs. nonconsumers = 1.07 (0.87-1.33), P_{trend} = 0.650), and also not for tree nut, peanut, or peanut butter consumption. In non-advanced prostate cancer cases, no significant association was found for total nuts (HR (95%CI) for 10+ g/day vs. nonconsumers = 1.12 (0.93-1.35), P_{trend} = 0.341), or for tree nuts or peanuts. However, peanut butter consumption was significantly associated with an increased risk of non-advanced prostate cancer in the multivariable-adjusted analysis (HR (95%CI) for 5+ g/day vs. nonconsumers = 1.33 (1.08-1.63), P_{trend} = 0.008), and in the continuous analysis (HR per 5 g/day increment (95%CI) = 1.09 (1.01-1.17)).

The associations between total nut consumption and total prostate cancer risk in strata of potential effect modifiers are presented in Table 3. The intake categories 5-<10 g/day and 10+ g/day were merged to increase statistical power. No significant interactions were observed for family history of prostate cancer, BMI, alcohol consumption, and level of education in the analyses for total (Table 3), advanced, and non-advanced prostate cancer (data not shown). Besides, no interactions were observed for peanut butter in non-advanced prostate cancer

Table 2. Age- and multivariable-adjusted HRs (95%CI) for total, advanced, and non-advanced prostate cancer according to nut consumption, the Netherlands Cohort Study, 1986-2006

Food item (g/day)	Median intake[a]	Person-years[b]	Total prostate cancer			Advanced prostate cancer			Non-advanced prostate cancer		
			Cases	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95%CI)[c]	Cases	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95%CI)[c]	Cases	Age-adjusted HR (95%CI)	Multivariable-adjusted HR (95%CI)[c]
Total nuts											
0	0.0	9393	1141	1.00 (ref)	1.00 (ref)	377	1.00 (ref)	1.00 (ref)	662	1.00 (ref)	1.00 (ref)
0.1-<5	2.5	10,023	1291	1.08 (0.93–1.25)	1.05 (0.91–1.22)	424	1.08 (0.89–1.30)	1.05 (0.87–1.27)	777	1.11 (0.95–1.31)	1.08 (0.91–1.27)
5-<10	8.5	4148	503	1.03 (0.85–1.25)	0.99 (0.81–1.20)	150	0.93 (0.73–1.20)	0.89 (0.69–1.15)	323	1.12 (0.91–1.38)	1.07 (0.86–1.33)
10+	21.4	7252	933	1.13 (0.96–1.32)	1.09 (0.92–1.29)	305	1.11 (0.91–1.36)	1.07 (0.87–1.33)	567	1.16 (0.97–1.38)	1.12 (0.93–1.35)
P-trend				0.244	0.409		0.435	0.650		0.185	0.341
Continuous per 5 g/day				1.01 (0.99–1.03)	1.01 (0.98–1.03)		1.01 (0.99–1.04)	1.01 (0.98–1.04)		1.01 (0.98–1.03)	1.00 (0.98–1.03)
Tree nuts											
0	0.0	22,309	2773	1.00 (ref)	1.00 (ref)	912	1.00 (ref)	1.00 (ref)	1655	1.00 (ref)	1.00 (ref)
0.1-<5	1.6	6963	886	1.04 (0.90–1.20)	1.00 (0.87–1.16)	284	1.02 (0.85–1.21)	1.00 (0.84–1.20)	539	1.05 (0.90–1.23)	1.00 (0.85–1.17)
5+	8.6	1544	209	1.11 (0.85–1.45)	1.10 (0.84–1.44)	60	0.97 (0.69–1.37)	0.97 (0.68–1.37)	135	1.19 (0.89–1.58)	1.17 (0.88–1.57)
P-trend				0.402	0.503		0.910	0.860		0.211	0.308
Continuous per 5 g/day				1.03 (0.95–1.13)	1.01 (0.93–1.11)		1.02 (0.92–1.14)	1.01 (0.90–1.13)		1.04 (0.95–1.14)	1.01 (0.92–1.11)
Peanuts											
0	0	10,550	1281	1.00 (ref)	1.00 (ref)	414	1.00 (ref)	1.00 (ref)	751	1.00 (ref)	1.00 (ref)
0.1-<5	2.5	10,952	1407	1.10 (0.96–1.26)	1.07 (0.93–1.23)	460	1.11 (0.93–1.33)	1.08 (0.90–1.30)	855	1.12 (0.96–1.31)	1.09 (0.93–1.28)
5-<10	8.5	3362	415	1.10 (0.89–1.35)	1.06 (0.86–1.31)	131	1.07 (0.83–1.39)	1.03 (0.79–1.35)	260	1.15 (0.92–1.44)	1.11 (0.89–1.40)
10+	21.4	5952	765	1.14 (0.96–1.35)	1.11 (0.93–1.33)	251	1.16 (0.94–1.43)	1.12 (0.89–1.40)	463	1.15 (0.96–1.39)	1.12 (0.93–1.37)
P-trend				0.219	0.332		0.291	0.454		0.226	0.352
Continuous per 5 g/day				1.01 (0.99–1.03)	1.01 (0.98–1.03)		1.01 (0.99–1.04)	1.01 (0.98–1.04)		1.00 (0.98–1.03)	1.00 (0.98–1.03)
Peanut butter											
0	0.0	22,148	2695	1.00 (ref)	1.00 (ref)	885	1.00 (ref)	1.00 (ref)	1593	1.00 (ref)	1.00 (ref)
0.1-<5	1.2	5290	701	1.15 (0.99–1.35)	1.14 (0.97–1.33)	229	1.14 (0.94–1.39)	1.14 (0.94–1.39)	424	1.18 (0.99–1.39)	1.15 (0.97–1.37)
5+	9.6	3379	472	1.19 (0.99–1.44)	1.19 (0.99–1.44)	142	1.09 (0.86–1.38)	1.08 (0.85–1.38)	312	1.33 (1.08–1.62)	1.33 (1.08–1.63)
P-trend				0.071	0.072		0.485	0.526		0.007	0.008
Continuous per 5 g/day				1.05 (0.98–.12)	1.06 (0.99–1.13)		1.02 (0.94–1.10)	1.02 (0.94–1.11)		1.08 (1.00–1.15)	1.09 (1.01–1.17)

[a] Median intake in the subcohort [b] Person-years in the subcohort [c] Adjusted for: age (years; continuous), family history of prostate cancer (yes/no), body mass index (<18.5/18.5-<25/25-<30/30+ kg/m^2), alcohol consumption (0/0.1-<5/5-<15/15-<30/≥30 g/day), level of education (primary or lower vocational/secondary or medium vocational/university or higher vocational), and total energy intake (kcal/day; continuous)

Table 3. Multivariable-adjusted[a] HRs (95%CI) for total prostate cancer according to total nut consumption in subgroups, the Netherlands Cohort Study, 1986-2006

		Total nut consumption (g/day)			P_{trend}	$P_{interaction}$
		0 g/day	0.1-<5 g/day	5+ g/day		
Overall						
	Cases/person-time at risk	1141/9393	1291/10023	1436/11400	0.688	
	HR (95%CI)	1.00 (reference)	1.05 (0.90–1.22)	1.05 (0.90-1.22)		
Family history of prostate cancer						0.114
Yes	Cases/person-time at risk	40/167	43/171	54/430	0.071	
	HR (95%CI)	1.00 (reference)	1.50 (0.52–4.36)	0.56 (0.20–1.53)		
No	Cases/person-time at risk	1101/9226	1248/9852	1382/10970	0.400	
	HR (95%CI)	1.00 (reference)	1.05 (0.90–1.22)	1.08 (0.93–1.26)		
Body mass index						0.149
18.5-<25 kg/m^2	Cases/person-time at risk	618/4726	681/5600	755/6116	0.982	
	HR (95%CI)	1.00 (reference)	0.91 (0.74–1.12)	0.96 (0.78–1.18)		
≥25 kg/m^2	Cases/person-time at risk	520/4634	608/4398	676/5271	0.534	
	HR (95%CI)	1.00 (reference)	1.24 (1.00–1.54)	1.15 (0.92–1.44)		
Alcohol consumption						0.805
0 g/day	Cases/person-time at risk	239/2236	142/1203	107/798	0.262	
	HR (95%CI)	1.00 (reference)	1.14 (0.79–1.65)	1.29 (0.84–1.99)		
0.1-<15 g/day	Cases/person-time at risk	505/4047	740/5544	646/5221	0.973	
	HR (95%CI)	1.00 (reference)	1.10 (0.88–1.36)	1.04 (0.83–1.29)		
≥15 g/day	Cases/person-time at risk	397/3109	409/3277	683/5380	0.833	
	HR (95%CI)	1.00 (reference)	0.96 (0.74–1.24)	1.01 (0.79–1.27)		
Level of education						0.055
Low	Cases/person-time at risk	538/4913	527/4482	507/3899	0.144	
	HR (95%CI)	1.00 (reference)	1.06 (0.85–1.31)	1.19 (0.94–1.49)		
Medium	Cases/person-time at risk	428/3017	476/3570	501/4599	0.045	
	HR (95%CI)	1.00 (reference)	0.98 (0.76–1.27)	0.80 (0.62–1.03)		
High	Cases/person-time at risk	175/1463	288/1970	428/2902	0.068	
	HR (95%CI)	1.00 (reference)	1.16 (0.80–1.67)	1.40 (0.98–1.99)		

[a] Adjusted for: age (years; continuous), family history of prostate cancer (yes/no), body mass index (18.5-<25/25-<30/30+ kg/m^2), alcohol consumption (0/0.1-<5/5-<15/15-<30/≥30 g/day), level of education (primary or lower vocational/secondary or medium vocational/university or higher vocational), and total energy intake (kcal/day; continuous)

cases (data not shown). In a sensitivity analysis, exclusion of the first two years of follow-up, exclusion of all nut and peanut butter consumers from the reference group, or mutual adjustment for the effects of other nut groups, did not importantly change the results (data not shown).

Discussion

We observed no significant associations between total nut, tree nut, and peanut consumption and total, advanced, and non-advanced prostate cancer risk. No association was observed for peanut butter in total and advanced prostate cancer cases, but higher intake of peanut butter was associated with a significantly increased risk of non-advanced prostate cancer.

No significant interactions with potential effect modifiers were observed with total nut consumption in total, advanced, and non-advanced prostate cancer cases.

To our knowledge, two cohort studies[20,21] and three case-control studies[22-24] have examined the association between nut consumption and prostate cancer risk. The large prospective Health Professionals Follow-Up Study and the Adventists Health Study both reported no significant associations between total nut consumption and total prostate cancer risk[20,21]. Thus, cohort studies consistently show no association between nut intake and prostate cancer risk, which is in line with our study. One case-control study also demonstrated no significant association between total nut consumption and total prostate cancer risk[22]. In contrast, two case-control studies demonstrated significantly decreased risks for total prostate cancer[23,24]. A random-effects meta-analysis (including one cohort study and four case-control studies; of which three studies have been mentioned above) demonstrated a decreased risk of total prostate cancer, albeit not statistically significant[16]. Two case-control studies that were included in the meta-analysis, but are not mentioned above, used different reference categories as they compared different diets, and combined the intake of nuts with different food groups. There are several limitations to this meta-analysis, including a high heterogeneity (I^2 = 59.7%) and the inclusion of case-control studies that are possibly prone to recall bias and selection bias. To our knowledge, no study has examined the differential effects of tree nuts, peanuts, and peanut butter.

Only two studies have examined the differential effects of total nut consumption on total prostate cancer and advanced prostate cancer. One case-control study demonstrated no significant association for aggressive prostate cancer[22]. The prospective Health Professionals Follow-up Study found no significant association for advanced prostate cancer and total nut consumption[20], which is consistent with our findings.

The increased risk of non-advanced prostate cancer for increased intake of peanut butter in our study is unexpected, as it is in contrast with the hypothesis that advanced prostate cancer cases have stronger associations with etiological factors. Furthermore, it is remarkable that an association was found for peanut butter consumption and not for peanut consumption. Additional analysis of lifestyle differences between consumers and nonconsumers of peanut butter in the subcohort did not indicate the potential for residual confounding (data not shown). Although we acknowledge that our estimates might be a result of chance findings, we tried to elucidate the mechanisms underlying the association. In 1986, in the year of exposure measurement, peanut butter contained more partially hydrogenated fatty acids (trans-fats) as compared to peanuts[15,30]. Several studies have indicated that trans-fatty acid markers are associated with an increased prostate cancer risk[31]. The intake of trans-fats increases systematic inflammation and insulin resistance, which both have been associated with prostate carcinogenesis[31]. The Physician's Health Study demonstrated that high blood concentrations of trans-fatty acids were unrelated to total or aggressive prostate cancer risk, but were associated with an increased risk of non-aggressive prostate tumors[31]. The SELECT study demonstrated that high blood levels of trans-fatty acids were associated with a reduced, albeit non-significant risk of high-grade prostate cancer, while a nonsignificant

increased risk of low-grade prostate cancer was demonstrated[32]. The specific mechanisms explaining why peanut butter increases the risk of non-advanced prostate cancer remain unclear. As our observations alternatively might be a result of chance findings, no firm conclusions can yet be drawn.

Strengths of the current study are the prospective design and the long follow-up with excellent retention. The potential for selection bias and information bias is therefore limited. Cases with inconsistent or incomplete dietary data, or cases with missing data on confounding variables were excluded from analysis, which might have introduced selection bias if the data were not missing completely at random. Furthermore, information bias might have been introduced because of self-reported baseline measurement. As exposure and confounders were only measured at baseline, exposure trends over time could not be detected, which might have introduced measurement error. We have no data on tree nut subtypes, and thus tree nuts were analysed as one group. Because not all tree nuts are comparable in nutritional composition[33], future studies should investigate the effect of specific types of tree nuts on the risk of prostate cancer.

In conclusion, we have demonstrated that the consumption of total nuts, tree nuts, and peanuts is not significantly associated with total, advanced, and non-advanced prostate cancer risk. However, peanut butter intake might be associated with an increased non-advanced prostate cancer risk. Further research is imperative to resolve the relation of peanut butter and non-advanced prostate cancer, and to determine the risk-benefit trade-offs associated with dietary intake of peanut butter.

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Chapter 9

Nut and peanut butter consumption and the risk of total cancer: A prospective cohort study

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Abstract

Background: Nut intake has been associated with reduced cancer-related mortality, but there is very limited evidence on total cancer risk. We investigated the associations of nut and peanut butter intake with the risk of total cancer and smoking- and alcohol-related cancer subgroups.

Methods: In the prospective Netherlands Cohort Study, 120,852 men and women aged 55-69 years provided information on lifestyle and dietary habits at baseline in 1986. After 20.3 years of follow-up, 19,255 total cancer cases and 3,499 subcohort members were included in multivariable-adjusted Cox regression analyses, using a case-cohort approach.

Results: No significant associations were found between total nut, tree nut, peanut, and peanut butter intake and total cancer risk in men and women. There were also no significant associations with smoking-(un)related and alcohol-(un)related cancers in both sexes.

Conclusion: Our findings suggest that nut and peanut butter intake are not associated with a reduced risk of total cancer in men or women.

Impact: Nut and peanut butter consumption are not related to the risk of total cancer.

Introduction

Growing scientific evidence has indicated that nut intake might have beneficial effects on cancer-related mortality and the risk of several cancer (sub)types, which might be related to their antioxidative and anti-inflammatory components (1). From a public health perspective, it is also relevant to know whether nut and peanut butter intake might contribute to a reduction of the total cancer burden. To our knowledge, this relation has only been investigated in one prospective cohort study, in which no associations were observed between nut intake and total cancer risk (2).

In this study, we investigated the associations of nut and peanut butter intake with the risk of total cancer in the prospective Netherlands Cohort Study (NLCS). These associations were also studied for smoking- and alcohol-related cancers, because the potential cancer preventive effects of nuts might be stronger for these carcinogen-related cancers.

Materials and Methods

At baseline in September 1986, 58,279 men and 62,573 women aged 55 to 69 years were included in the NLCS (3). All participants filled in a self-administered baseline questionnaire on potential cancer risk factors. A case-cohort approach was applied, in which cases were derived from the entire cohort and person-time at risk from a subcohort consisting of 5,000 randomly sampled participants. Follow-up of cancer incidence was performed through annual record linkage with the Netherlands Cancer Registry and the Netherlands Pathology Registry (PALGA).

After 20.3 years of follow-up, we included 19,255 cancer cases (of which 765 in the subcohort) and 3,499 subcohort members after excluding participant with prevalent cancer (excluding skin cancer), incomplete or inconsistent dietary data, missing data on exposures and confounders, and cases with noninvasive tumors or without microscopic confirmation. Smoking-related cancers comprised cancers of the oral cavity (including lip), pharynx, esophagus, stomach, colorectum, liver, pancreas, nasal cavity, paranasal sinuses, larynx, trachea, lung, uterine cervix, ovary, kidney, ureter, urinary bladder, and myeloid leukemia (4). Alcohol-related cancers included cancers of the oral cavity (including lip), pharynx, esophageal squamous cell carcinoma, colorectum, liver, larynx, and breast (4). The smoking- and alcohol-unrelated cancer subgroups included all cancers not described above.

Habitual diet was assessed using a validated 150-item self-administered semiquantitative food frequency questionnaire (FFQ). Nut and peanut butter intake were measured as described previously (5).

Cox proportional hazards models were used to estimate multivariable-adjusted hazard ratios (HR) and 95% confidence intervals (CI) for the associations between total nut intake and total cancer risk. Associations were also examined for tree nut, peanut, and peanut

Table 1. Baseline characteristics (mean (SD) or %) of subcohort members and total cancer cases; the NLCS, 1986-2006

	Subcohort	Total cancer	Smoking-related cancer	Smoking-unrelated cancer	Alcohol-related cancer	Alcohol-unrelated cancer
Men						
N	1834	12,184	6723	5461	2385	9799
Age (years)	61.2 (4.2)	61.6 (4.1)	61.6 (4.1)	61.6 (4.1)	61.5 (4.1)	61.6 (4.1)
Body Mass Index (kg/m^2)	24.9 (2.6)	25.0 (2.6)	25.1 (2.7)	25.0 (2.5)	25.2 (2.7)	25.0 (2.6)
Height (cm)	176.6 (6.6)	176.7 (6.7)	176.6 (6.7)	176.7 (6.7)	176.7 (6.6)	176.7 (6.7)
Ever cigarette smoker (%)	86.4	88.8	91.7	85.2	88.3	88.9
University or higher vocational education (%)	20.3	20.0	17.7	22.8	20.4	19.9
Nonoccupational physical activity (min/day)	81.0 (67.4)	80.8 (65.7)	81.0 (67.4)	80.5 (63.4)	81.9 (67.0)	80.5 (65.3)
Family history of cancer (%)	45.6	48.6	48.8	48.2	49.5	48.3
Daily energy intake (kcal)	2167 (499)	2166 (492)	2170 (495)	2161 (487)	2153 (487)	2169 (493)
Total nut intake (g/day)	7.9 (13.7)	7.9 (14.4)	7.4 (13.5)	8.4 (15.4)	7.6 (12.9)	7.9 (14.7)
Tree nut intake (g/day)	1.0 (3.4)	1.0 (3.6)	0.9 (3.1)	1.1 (4.2)	1.0 (3.4)	1.0 (3.7)
Peanut intake (g/day)	6.9 (13.0)	6.8 (13.2)	6.5 (12.6)	7.2 (13.8)	6.6 (12.1)	6.9 (13.4)
Peanut butter intake (g/day)	1.4 (4.2)	1.5 (4.3)	1.4 (4.2)	1.6 (4.4)	1.6 (4.3)	1.5 (4.3)
Alcohol intake (g/day)	15.1 (17.1)	16.7 (17.7)	17.8 (18.8)	15.2 (16.1)	18.0 (19.4)	16.3 (17.2)
Women						
N	1665	7071	3013	4058	3756	3315
Age (years)	61.3 (4.2)	61.5 (4.1)	61.7 (4.1)	61.4 (4.1)	61.5 (4.1)	61.6 (4.2)
Body Mass Index (kg/m^2)	25.0 (3.5)	25.2 (3.6)	24.9 (3.6)	25.3 (3.5)	25.2 (3.5)	25.2 (3.7)
Height (cm)	165.3 (6.1)	165.8 (6.4)	165.9 (6.3)	165.8 (6.4)	165.9 (6.4)	165.7 (6.3)
Ever cigarette smoker (%)	41.4	43.9	47.4	41.2	43.4	44.4
University or higher vocational education (%)	9.8	9.7	9.7	9.7	10.1	9.3
Nonoccupational physical activity (min/day)	66.1 (50.4)	63.1 (51.0)	64.2 (52.9)	62.4 (49.6)	62.4 (50.7)	64.0 (51.4)
Family history of cancer (%)	47.9	52.3	52.1	52.4	53.1	51.4
Age at menarche (years)	13.7 (1.8)	13.5 (1.7)	13.5 (1.7)	13.5 (1.7)	13.5 (1.7)	13.6 (1.7)
Age at menopause (years)	48.8 (4.4)	49.0 (4.3)	48.7 (4.4)	49.2 (4.3)	49.0 (4.3)	49.0 (4.3)
Parous (%)	81.7	80.8	81.3	80.4	80.9	80.7
Age at first birth (in parous, years)	27.0 (4.2)	27.0 (4.2)	26.9 (4.2)	27.1 (4.2)	27.2 (4.2)	26.9 (4.3)
Number of children (in parous, n)	3.4 (1.9)	3.3 (1.8)	3.3 (1.9)	3.2 (1.8)	3.3 (1.8)	3.3 (1.8)
Oral contraceptive use (%)	25.3	24.3	25.2	23.6	25.1	23.4
Hormone replacement therapy (%)	13.5	13.3	12.9	13.6	13.5	13.1

(Continued)	Subcohort	Total cancer	Smoking-related cancer	Smoking-unrelated cancer	Alcohol-related cancer	Alcohol-unrelated cancer
Daily energy intake (kcal)	1689 (391)	1689 (386)	1687 (381)	1691 (389)	1688 (389)	1690 (382)
Total nut intake (g/day)	4.5 (8.6)	4.4 (8.8)	4.2 (8.6)	4.5 (8.9)	4.4 (9.0)	4.4 (8.5)
Tree nut intake (g/day)	1.1 (4.1)	1.0 (3.2)	1.0 (3.1)	1.0 (3.3)	1.0 (3.3)	1.0 (3.1)
Peanut intake (g/day)	3.4 (7.0)	3.4 (7.4)	3.2 (7.3)	3.5 (7.5)	3.4 (7.6)	3.4 (7.3)
Peanut butter intake (g/day)	1.2 (3.5)	1.1 (3.2)	1.0 (3.3)	1.1 (3.2)	1.1 (3.2)	1.1 (3.3)
Alcohol intake (g/day)	6.0 (9.6)	6.7 (11.1)	6.9 (11.6)	6.5 (10.7)	6.9 (11.4)	6.4 (10.7)

Table 2. Multivariable-adjusted HRs and 95% CI for total cancer and for smoking-(un)related and alcohol-(un)related cancers, according to nut and peanut butter consumption; the NLCS, 1986-2006

			Total cancer		Smoking-related cancer		Smoking-unrelated cancer		Alcohol-related cancer		Alcohol-unrelated cancer	
	Median intake[a]	Person-years	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)
Men												
Total nut intake (g/day)												
0	0.0	8231	3889	1.00 (reference)	2292	1.00 (reference)	1597	1.00 (reference)	761	1.00 (reference)	3128	1.00 (reference)
0.1-<5	2.5	8972	3990	0.96 (0.84-1.11)	2175	0.94 (0.80-1.09)	1815	1.01 (0.86-1.17)	789	0.96 (0.81-1.14)	3201	0.97 (0.83-1.12)
5-<10	8.5	3679	1527	0.91 (0.75-1.09)	816	0.88 (0.72-1.08)	711	0.95 (0.78-1.16)	305	0.90 (0.71-1.12)	1222	0.91 (0.75-1.10)
10+	21.4	6496	2778	0.96 (0.82-1.13)	1440	0.88 (0.74-1.05)	1338	1.08 (0.91-1.28)	530	0.91 (0.75-1.11)	2248	0.97 (0.83-1.14)
p-trend				0.69		0.18		0.38		0.38		0.82
Continuous, per 5 g/day increment				1.00 (0.98-1.02)		0.99 (0.96-1.01)		1.01 (0.99-1.03)		0.99 (0.96-1.01)		1.00 (0.98-1.02)
p-heterogeneity								<0.001				0.88
Tree nuts (g/day)												
0	0.0	19756	8951	1.00 (reference)	5056	1.00 (reference)	3895	1.00 (reference)	1754	1.00 (reference)	7197	1.00 (reference)
0.1-<5	1.6	6251	2660	0.98 (0.85-1.12)	1385	0.95 (0.82-1.10)	1275	1.00 (0.87-1.16)	514	0.95 (0.81-1.12)	2146	0.98 (0.85-1.13)
5+	8.9	1372	573	1.02 (0.79-1.31)	282	0.95 (0.72-1.26)	291	1.08 (0.83-1.42)	117	1.03 (0.76-1.40)	456	1.01 (0.78-1.31)
p-trend				0.98		0.60		0.58		0.96		0.99
Continuous, per 5 g/day increment				1.01 (0.94-1.10)		1.00 (0.91-1.09)		1.02 (0.93-1.12)		1.01 (0.92-1.11)		1.01 (0.93-1.10)
p-heterogeneity								<0.001				0.81
Peanuts (g/day)												
0	0.0	9277	4311	1.00 (reference)	2524	1.00 (reference)	1787	1.00 (reference)	862	1.00 (reference)	3449	1.00 (reference)
0.1-<5	2.5	9777	4321	0.98 (0.86-1.12)	2332	0.94 (0.81-1.08)	1989	1.05 (0.91-1.22)	847	0.94 (0.80-1.11)	3474	0.99 (0.86-1.14)
5-<10	8.5	2978	1249	0.95 (0.77-1.16)	655	0.87 (0.70-1.08)	594	1.07 (0.86-1.32)	241	0.90 (0.71-1.15)	1008	0.96 (0.78-1.17)
10+	21.4	5346	2303	0.98 (0.83-1.16)	1212	0.91 (0.76-1.09)	1091	1.10 (0.92-1.31)	435	0.90 (0.73-1.09)	1868	1.01 (0.85-1.19)
p-trend				0.87		0.34		0.37		0.33		0.93
Continuous, per 5 g/day increment				1.00 (0.97-1.02)		0.99 (0.96-1.01)		1.01 (0.98-1.03)		0.99 (0.96-1.01)		1.00 (0.97-1.02)
p-heterogeneity								<0.001				0.71
Peanut butter (g/day)												
0	0.0	19616	8687	1.00 (reference)	4891	1.00 (reference)	3796	1.00 (reference)	1725	1.00 (reference)	6962	1.00 (reference)
0.1-<5	1.2	4696	2095	1.09 (0.94-1.27)	1086	1.03 (0.88-1.21)	1009	1.17 (1.00-1.37)	374	0.98 (0.82-1.18)	1721	1.12 (0.96-1.30)

(Continued)			Total cancer		Smoking-related cancer		Smoking-unrelated cancer		Alcohol-related cancer		Alcohol-unrelated cancer	
	Median intake[a]	Person-years	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)
5+	9.6	3066	1402	1.09 (0.91-1.31)	746	1.05 (0.86-1.28)	656	1.14 (0.94-1.38)	286	1.15 (0.93-1.42)	1116	1.08 (0.90-1.30)
p-trend				0.35		0.66		0.19		0.21		0.44
Continuous, per 5 g/day increment				1.05 (0.99-1.13)		1.05 (0.97-1.13)		1.06 (0.99-1.14)		1.08 (1.00-1.17)		1.05 (0.98-1.12)
p-heterogeneity								<0.001				0.07
Women												
Total nut intake (g/day)												
0	0.0	11028	2865	1.00 (reference)	1268	1.00 (reference)	1597	1.00 (reference)	1533	1.00 (reference)	1332	1.00 (reference)
0.1-<5	2.1	10290	2615	1.03 (0.90-1.18)	1101	1.02 (0.88-1.19)	1514	1.04 (0.90-1.20)	1389	1.00 (0.87-1.16)	1226	1.06 (0.92-1.23)
5-<10	7.8	2942	720	1.05 (0.85-1.29)	294	1.01 (0.80-1.28)	426	1.08 (0.86-1.34)	377	0.98 (0.79-1.23)	343	1.12 (0.89-1.41)
10+	15.7	3797	871	0.89 (0.74-1.08)	350	0.83 (0.66-1.03)	521	0.94 (0.77-1.16)	457	0.85 (0.69-1.04)	414	0.95 (0.77-1.18)
p-trend				0.26		0.09		0.59		0.11		0.66
Continuous, per 5 g/day increment				0.99 (0.96-1.03)		0.98 (0.94-1.02)		1.00 (0.96-1.04)		0.99 (0.95-1.03)		1.00 (0.96-1.03)
p-heterogeneity								0.07				0.91
Tree nuts (g/day)												
0	0.0	19752	5030	1.00 (reference)	2178	1.00 (reference)	2852	1.00 (reference)	2688	1.00 (reference)	2342	1.00 (reference)
0.1-<5	1.6	6686	1712	1.05 (0.91-1.20)	692	1.02 (0.87-1.19)	1020	1.07 (0.92-1.24)	891	0.99 (0.85-1.16)	821	1.11 (0.95-1.29)
5+	8.9	1619	329	0.83 (0.64-1.08)	143	0.85 (0.63-1.15)	186	0.82 (0.62-1.08)	177	0.82 (0.62-1.08)	152	0.86 (0.64-1.14)
p-trend				0.24		0.34		0.23		0.17		0.44
Continuous, per 5 g/day increment				0.97 (0.90-1.05)		0.96 (0.87-1.06)		0.97 (0.90-1.05)		0.96 (0.88-1.05)		0.98 (0.90-1.06)
p-heterogeneity								0.09				0.57
Peanuts (g/day)												
0	0.0	13081	3363	1.00 (reference)	1473	1.00 (reference)	1890	1.00 (reference)	1781	1.00 (reference)	1582	1.00 (reference)
0.1-<5	2.0	10269	2593	1.04 (0.91-1.18)	1088	1.03 (0.89-1.19)	1505	1.04 (0.91-1.20)	1395	1.03 (0.90-1.19)	1198	1.05 (0.91-1.21)
5-<10	8.5	2108	502	0.97 (0.77-1.23)	205	0.95 (0.72-1.24)	297	1.00 (0.78-1.28)	263	0.94 (0.73-1.21)	239	1.01 (0.78-1.31)
10+	17.1	2598	613	0.93 (0.75-1.15)	247	0.86 (0.67-1.11)	366	0.98 (0.78-1.23)	317	0.88 (0.69-1.11)	296	0.99 (0.78-1.26)
p-trend				0.43		0.21		0.75		0.21		0.88
Continuous, per 5 g/day increment				1.00 (0.96-1.04)		0.98 (0.94-1.03)		1.01 (0.96-1.05)		0.99 (0.95-1.04)		1.00 (0.95-1.05)
p-heterogeneity								0.20				0.79
Peanut butter (g/day)												
0	0.0	20261	5166	1.00 (reference)	2212	1.00 (reference)	2954	1.00 (reference)	2757	1.00 (reference)	2409	1.00 (reference)

(Continued)			Total cancer		Smoking-related cancer		Smoking-unrelated cancer		Alcohol-related cancer		Alcoohol-unrelated cancer	
	Median intake[a]	Person-years	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)	Cases	Multivariable-adjusted[b] HR (95% CI)
0.1-<5	1.2	5092	1285	1.04 (0.90-1.22)	550	1.05 (0.88-1.25)	735	1.04 (0.88-1.22)	672	1.03 (0.87-1.21)	613	1.06 (0.90-1.25)
5+	5.3	2703	620	0.96 (0.78-1.18)	251	0.93 (0.74-1.18)	369	0.98 (0.79-1.22)	327	0.95 (0.76-1.18)	293	0.97 (0.77-1.21)
p-trend				0.76		0.63		0.91		0.70		0.86
Continuous, per 5 g/day increment				0.96 (0.88-1.05)		0.95 (0.86-1.05)		0.98 (0.89-1.07)		0.95 (0.86-1.04)		0.98 (0.89-1.07)
p-heterogeneity								0.50				0.76

[a] Median intake (g/day) in the subcohort

[b] Adjusted for age (years; continuous), cigarette smoking (status (never, former, current), frequency (n/day; continuous, centered), and duration (years; continuous, centered)), BMI (<18.5, 18.5-<25, 25-<30, ≥30 kg/m^2), height (cm; continuous), nonoccupational physical activity (≤30, >30-60, >60-90, >90 min/day), daily energy intake (kcal/day; continuous), alcohol intake (0, 0.1-<5, 5-<15, 15-<30, 30+ g/day), educational level (low, medium, high), family history of cancer (no, yes), age at menarche (years; continuous; in women only), age at menopause (years; continuous; in women only), parity and age at first child birth (nulliparous, 1-2 children - <25 years, 1-2 children - ≥25 years, ≥3 children - <25 years, ≥3 children - ≥25 years; in women only), oral contraceptive use (never, ever; in women only), hormone replacement therapy use (never, ever; in women only)

butter intake, and for smoking- and alcohol-(un)related cancers. Standard errors (SE) were calculated using a robust Huber-White sandwich estimator (6). Scaled Schoenfeld residuals, -ln(-ln) survival plots, and time-varying covariates confirmed that assuming proportional hazards was appropriate. Heterogeneity between the smoking- and alcohol-(un)related subgroups was tested using a competing risk procedure that applies a bootstrapping method to estimate SEs in case-cohort designs.

Results

Mean total nut intake in the subcohort was 7.9 g/day in men and 4.5 g/day in women. Baseline characteristics are presented in Table 1. We observed no associations between total nut, tree nut, peanut, and peanut butter intake and total cancer risk in men or women (Table 2). Also no associations were found for smoking- and alcohol-(un)related cancers, except for a borderline significant positive association between 0.1-<5 g peanut butter intake/day and smoking-unrelated cancer in men (HR (95% CI) = 1.17 (1.00-1.37)).

Heterogeneity tests between smoking-related and smoking-unrelated cancers were significant for all nut variables in men (*P*-heterogeneity <0.001). This is probably the result of the large number of cases and the opposite directions of the nonsignificant associations in the smoking-related and smoking-unrelated subgroups in men.

Discussion

Our results are in line with the only other cohort study that investigated the relation between nut intake and total cancer risk, in which also no significant associations were observed (2). Prospective evidence on total cancer is thus very limited. In a recent meta-analysis, a significantly reduced "overall" cancer risk was observed after pooling 33 prospective cohort studies that investigated the relations between nut intake and several cancer (sub)types (7). However, this pooled estimate is not informative, because it does not include all cancer types and might be affected by publication bias.

Here, we presented results for total cancer, but associations for several cancer (sub)types have been investigated previously in the NLCS, for example ref. (5). Strengths of the NLCS include the prospective design, the inclusion of all cancer types (except skin cancer), the large number of cases, and the long and complete follow-up of 20.3 years. Unfortunately, we only have baseline measurements. Nevertheless, the FFQ showed adequate reproducibility (8) and the NLCS consists of an older population with relatively stable dietary habits. Furthermore, we cannot completely exclude residual confounding by (un)measured factors.

In conclusion, the results of this prospective cohort study do no support the hypothesis that nut intake is associated with a reduced total cancer risk in men or women.

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Chapter 10

General discussion

Summary of main findings

The aim of this thesis was to investigate the associations between total nut, tree nut, peanut, and peanut butter intake and the risk of several cancers and their subtypes in men and women in the NLCS. Moreover, we investigated whether these associations are nonlinear and whether they are modified by sex, alcohol intake, smoking, BMI, physical activity, and educational level.

Increased nut intake was associated with a significantly decreased risk of esophageal squamous cell carcinoma and gastric non-cardia adenocarcinoma (Chapter 2). A nonsignificant positive association was seen for the risk of esophageal adenocarcinoma, and no clear association was found for gastric cardia adenocarcinoma. For peanut butter consumption, nonsignificant positive associations were found for the esophageal cancer subtypes, but not for the gastric cancer subtypes.

In Chapter 3, we observed significant nonlinear inverse associations with rectal cancer for total nut, peanut, and peanut butter intake in women in restricted cubic spline analyses, and borderline significant nonlinear inverse associations for total nut and peanut intake in men. No significant associations were observed for other anatomical CRC subtypes. Regarding the molecular CRC subtypes after 7.3 years of follow-up, peanut butter intake was significantly associated with an increased risk of colorectal tumors that did not develop through the serrated neoplasia pathway in men.

Nut intake was non-significantly inversely associated with microscopically confirmed pancreatic cancer risk in men, and a significantly reduced risk was observed for peanut butter intake (Chapter 4). In women, no significant associations with microscopically confirmed pancreatic cancer were seen for nut or peanut butter intake. The observed associations were weaker when looking at total pancreatic cancer risk.

In Chapter 5, total nut, tree nut, and peanut intake were significantly related to a reduced risk of small cell lung carcinoma in men, after controlling for detailed smoking habits. No significant associations were found in men for the other lung cancer subtypes or total lung cancer, in women, or for peanut butter intake.

A significant inverse association was found between nut intake and the risk of estrogen receptor (ER)-negative postmenopausal breast cancer (Chapter 6). There were no significant inverse associations with ER-positive or total breast cancer risk. There was no variation in associations between progesterone receptor (PR) subtypes, although the ER-negative PR-negative subtype was also significantly inversely associated with nut intake. No significant associations were found between peanut butter intake and breast cancer risk or its ER/PR-subtypes.

In Chapter 7, no significant associations between the intake of total nuts, tree nuts, peanuts, and peanut butter and the risk of endometrial or ovarian cancer were observed. Significant interactions were seen in the endometrial cancer analyses. In particular, an interesting interaction between total nut intake and cigarette smoking status was observed, in which increasing nut consumption appeared to attenuate the inverse association between cigarette smoking and endometrial cancer risk.

No significant associations were seen between total nut, tree nut, and peanut intake and the risk of total, advanced, and non-advanced prostate cancer (Chapter 8). Peanut butter consumption was significantly positively associated with the risk of non-advanced prostate cancer.

Finally, in Chapter 9, total nut, tree nut, peanut, and peanut butter intake were not associated with the risk of total cancer in men or women. Moreover, no associations were found with smoking-(un)related and alcohol-(un)related cancers in both sexes.

Overall interpretation of the study results

In general, the (non)significant inverse associations for tree nut intake in relation to cancer risk appeared to be somewhat stronger than the associations for total nut and peanut intake. The mean tree nut intake (1.0 g/day in men and 1.1 g/day in women) in the NLCS is much lower than the mean peanut intake (6.9 g/day in men and 3.3 g/day in women), and therefore constitutes a smaller part of the total nut intake (7.9 g/day in men and 4.4 g/day in women). Consequently, the observed associations for total nut intake strongly resemble those for peanut intake. Furthermore, as described previously, the nutrient composition of tree nuts and peanuts is quite similar (1, 2). However, peanuts contain more proteins than for example almonds, cashew nuts, hazelnuts, and walnuts (1, 2). Moreover, peanuts contain more mono-unsaturated fatty acids, niacin, and potassium than walnuts (1, 2), while the antioxidant capacity of walnuts is higher (3, 4). Moreover, tree nuts represent a greater variety of nut types, while peanuts only include one food item. Therefore, the diversity and variation in beneficial nutrients is higher for tree nuts than for peanuts.

For peanut butter, the associations with cancer risk were inconsistent. Significant inverse associations with peanut butter intake were seen for microscopically confirmed pancreatic cancer (in men) and rectal cancer (in women), and a significant positive association with non-advanced prostate cancer. Several positive, but nonsignificant associations were found for the esophageal cancer subtypes, and for colorectal cancer that did not develop through the serrated neoplasia pathway (in men). The nonsignificant positive associations with the esophageal cancer subtypes were tested in men and women combined in categorical analyses, but in continuous analyses these positive associations were particularly seen in men. The (non)significant positive associations for peanut butter intake were thus mainly found in men.

As mentioned in the Introduction (Chapter 1), peanut butter that was produced in the Netherlands in (the years preceding) 1986 contained less niacin, and more vitamin B6, sodium, and partially hydrogenated fatty acids than peanuts (1). These latter components were added to improve the quality, taste, and presentation of peanut butter. Consequently, because of the different nutrient composition of nuts and peanut butter, associations with cancer risk may also differ between these food items. For example, trans fatty acids have been hypothesized to increase cancer risk (5, 6).

Sex differences in the associations between nut or peanut butter intake and the risk of several cancer (sub)types were observed for microscopically confirmed pancreatic cancer, rectal cancer, and small cell lung carcinoma. It is unclear why these sex differences do exist, although they might be related to differences in the average nut and peanut butter intake. Men consume on average more nuts and peanut butter than women (7.9 g/day versus 4.5 g/day for total nut intake and 1.4 g/day versus 1.2 g/day for peanut butter intake). However, for rectal cancer, we observed a significant inverse association in women and not in men. Therefore, this explanation is unlikely to account for all observed differences. In addition, nuts are a source of phytoestrogens, and these hormonal components and related hormonal mechanisms might contribute to the observed sex differences (7, 8). However, we did not find associations between nut and peanut butter intake and hormone dependent cancers, such as endometrial, ovarian, and ER-positive breast cancer. Although we might thus not be able to clarify the observed differences between men and women, these observations provide a basis for further research and require confirmation in other studies.

We also found that the associations of total nut, tree nut, peanut, and peanut butter with the risk of cancer were often nonlinear. For example, nonlinear relations were observed for gastric non-cardia adenocarcinoma, microscopically confirmed pancreatic cancer, ER-negative breast cancer, and rectal cancer (women). Nonlinear relations were expected because a clear deviation from linearity was also observed for the relation between nut consumption and cancer mortality in a dose-response meta-analysis (9). Moreover, in nutritional epidemiologic research, linear dose-response relations are not very common, because the capacity for absorbing, transporting, metabolizing and/or storing most dietary factors is often limited (10). Moreover, enzyme activity, which often regulates the effects of dietary factors, can become saturated (10). As a result, there might be a clear leveling-off in the relation between a dietary factor and outcome of interest.

Methodological considerations

Our study has several important strengths and limitations. Some of the methodological considerations will be described below.

The most important strengths of our study include the prospective nature, long duration, and complete follow-up of the NLCS. These assets make selection bias, recall bias, reverse causation, and bias related to loss to follow-up unlikely. Moreover, because of the large

number of participants in the NLCS and the long follow-up, the number of cancer cases was very large. This enabled us to investigate the associations between nut and peanut butter intake and the risk of rarer cancer (sub)types.

In 1986 (the starting year of the NLCS), the beneficial health effects of nut consumption on, amongst others, cardiovascular diseases and cancer were unlikely to be known in the general public. As described in the Introduction (Chapter 1), the interest in nuts started with a publication of the Adventist Health Study in 1992 (11). Therefore, it is not likely that nuts were consumed because they were considered as a healthy food item at the start of the NLCS. Also, selective reporting of the nut and peanut butter intake is hence unlikely in our study. Misclassification of nut consumption still might have occurred, but is unlikely to be differential.

Despite the fact that the beneficial health effects of nuts were not known in 1986, nut consumers in the NLCS were found to have an overall healthier lifestyle than nonconsumers. Nut consumers were on average younger, leaner (in women), ate more fruits and vegetables, were less often hypertensive, more often higher educated, and more often used nutritional supplements than nonconsumers (9, 12). Moreover, female nut consumers more often had a late age at first child birth, and more often used oral contraceptives and postmenopausal hormone replacement therapy (HRT) (12). In contrast to these healthier habits, nut consumers drank on average more alcohol and were more often ever smokers than nonconsumers. In several other cohort studies, a similar overall healthier lifestyle pattern was seen among nut consumers (13-18).Unlike the nut consumers in the NLCS, nut consumers in several other cohort studies were less likely to smoke tobacco (13, 15-18). We also observed a healthier lifestyle pattern among peanut butter consumers than in nonconsumers in the NLCS: peanut butter consumers were on average younger, leaner, drank less alcohol, were more often never smokers (women), higher educated, more often used nutritional supplements, and scored higher on the adapted Mediterranean diet score (9, 12). Moreover, female peanut butter consumers more often had a late age at first child birth, more often used oral contraceptives, and less often used postmenopausal HRT (12). In the NIH-AARP Diet and Health Study, the lifestyle pattern among peanut butter consumers differed from our study: peanut butter consumers in the NIH-AARP were more likely to be smokers, more physically active, to have a higher BMI, consume less alcohol, have less education, and were less likely to have a history of heart disease and hypertension than nonconsumers (13). Most of the above-mentioned factors have also been associated with the risk of cancer and may therefore be confounding factors in the relation between nut consumption and cancer risk. We extensively corrected for these potential confounders and still observed inverse associations for several cancer (sub)types. Nevertheless, we cannot completely exclude residual confounding by measured and unmeasured confounders. It is not clear in which direction this bias would point because of the complexity and interrelationship of these factors.

Another important limitation of our study is that habitual dietary intakes were only measured with a food frequency questionnaire (FFQ) at baseline in 1986, while these might

have changed over time. Nevertheless, as described in previous chapters, dietary habits in our cohort appeared to be stable for at least five years in a reproducibility study (19), nut intake appeared to be relatively constant in another prospective cohort with repeated measurements (20), and our study population consisted of an older population in which dietary habits are relatively stable.

Concerning nut and peanut butter intake, three food items were included in the FFQ: 1) peanuts, 2) other, mixed nuts (tree nuts), and 3) peanut butter. We have no data indicating which nut types were consumed exactly. However, walnuts and hazelnuts have been grown and consumed in the Netherlands as far back as in Roman times (21). Moreover, according to trade data from the Food and Agriculture Organization (FAO) of the United Nations, peanuts, almonds, walnuts, and cashew nuts have been imported in the Netherlands since at least 1961, although the import of cashew nuts was still relatively limited at that time (22). No trade data from the FAO are available before 1961. Between 1961 and 1986 the import of these nut types gradually increased, and since 1977 hazelnuts have been imported as well (22). In 1986, the starting year of the NLCS, the above-mentioned nut types were still the main nut types that were imported in the Netherlands (22). The import of other nut types, such as pistachios, Brazil nuts, and other nuts (pecan nuts, macadamia nuts, and pine nuts) was still very low that year, but has gradually increased since the year 1990 (22). Obviously, these import data do not directly represent the (un)availability of specific nut types to consumers. Nevertheless, it gives an indication that the availability of nuts has become more diverse and extensive during the 20.3 years of follow-up in our study and during the lifetime of NLCS participants. Concerning peanut butter, one of the biggest producers in the Netherlands started with the production and marketing of peanut butter in 1948, after the French Calvé brothers had brought peanut butter from the United States to the Netherlands (23-25). In the aftermath of the second world war, this relatively cheap and very nutrient dense product quickly became popular (25).

NLCS participants were born between the years 1916 and 1932 and thus aged 16 to 32 years when peanut butter was first marketed in the Netherlands in 1948 (23-25). Consequently, participants were not exposed to peanut butter during childhood and early adolescence, while nuts could have been consumed throughout their lives. For some cancer types, it has been observed that exposures during adolescence have a stronger influence on cancer development than adult exposures. Such an association for nut intake during adolescence has been found for the risk of benign breast disease and breast cancer (26, 27). Whether this might also be the case for peanut butter is unclear.

The nutrient content of peanut butter has also changed over the follow-up period. For example, peanut butter that was sold in the Netherlands at baseline of the NLCS in 1986 contained trans fatty acids (1, 9). Trans fatty acids have often been used in food manufacturing because of their semi-solidity that enhances the palatability of foods, their stability during deep-frying, and because they increase the shelf life of products (6, 28). However, research has shown that the consumption of trans fatty acid might increase the risk of coronary heart disease, diabetes, and potentially cancer (5, 6, 28). Consequently, the amount of trans fatty

acids in foods have dropped to very low levels in the Netherlands since the start of the 21st century after publications and collaborations of the Dutch Health Council and the Dutch Task Force for the improvement of the Fatty Acid Composition (29, 30).

Another constraint of our study, and of many other cohort studies, is that no data on the processing of nuts are available. Nuts can be eaten raw, but are mostly eaten after being roasted, stir-fried, oil-fried, or boiled (31, 32). Walnuts are an exception to this, because they are mainly eaten as a natural, raw, and unpeeled product (31, 32). Nuts can be roasted with or without skin. To remove the skin of a nut, a nut can be blanched followed by a peeling step (31, 32). Blanching, removal of the skin, and roasting might decrease the total phenolic content and antioxidant capacity of the nut. Roasting might also increase the total phenolic content and antioxidant capacity by the release and formation of phenolic compounds (31, 32). Also, salt might be added, which might also influence the overall effect of nuts on the risk of cancer.

Another methodological issue that we encountered was the difficulty of comparing our results to the results from other studies. First, in many studies, nut intake was combined with the intake of other food items, like fruits, legumes, pulses, beans, lentils, or tasty snacks. This makes it difficult to draw conclusions about nut intake itself, but also makes the comparison of studies with different exposure variables very hard. Secondly, the results of case-control studies on the relation between nut intake and cancer risk appeared generally to be stronger and more often significant than those of cohort studies. This might be the result of selection and information biases intrinsic to case-control studies. Moreover, some studies reported absolute average daily nut intakes, while others reported average intake frequencies or only quantiles of intake. Consequently, studies can be very difficult to compare as the intake quantities can be on completely different positions on the total range of nut consumption.

In contrast to what is often thought, the average intake of nuts and particularly peanuts is not so low in the Netherlands. According to the EPIC cohort, the population mean intake level of peanuts was higher in the Netherlands than in the nine other participating European countries in the EPIC cohort, and the average tree nut intake in the Netherlands ranked fourth, after France, Spain, and the United Kingdom (33). In addition, the average total nut intake in the NLCS was 7.9 g/day (mean) and 2.8 g/day (median) in men and 4.5 g/day (mean) and 1.6 g/day (median) in women (Chapter 9). This is higher than the mean total nut intake in the NIH-AARP cohort study in the United States (20.14 g/week = 2.88 g/day; men and women combined) and higher than the median total nut intake in the Golestan Cohort Study in Iran (1.89 g/day; men and women combined) (16, 34).

Recommendations for future research

Based on the results of this thesis, we recommend future research in the field of nut and peanut butter consumption and cancer risk to focus on the following aspects.

Firstly, we recommend to replicate the findings in this thesis in other prospective cohort studies, because the prospective evidence on the associations between nut intake and cancer risk is still limited. Especially for rarer cancer types, replication of our study results in prospective cohort studies with more cases is important. In addition, the prospective evidence for a relation between peanut butter and cancer risk is even more limited at this moment. Therefore, we also advise to replicate our findings for peanut butter in other prospective cohort studies.

We also recommend to further investigate the possible mechanisms underlying the observed associations between nut and peanut butter consumption and cancer risk. At this moment, several potential mechanisms have been hypothesized, but there is no clear evidence which, if any, genuinely contribute to the prevention of cancer. This information may also contribute to a better understanding of why inverse associations were seen for certain cancer (sub)types, but not for others. Molecular epidemiological research might also help to this purpose. Unfortunately, we could only use a molecular-epidemiological approach for colorectal cancer with a limited number of cases and for only a limited number of genes. Nevertheless, these techniques might be used to further elucidate the underlying mechanisms, especially because the field of molecular epidemiology is dynamic and continuously developing with regard to markers and analyses techniques.

Thirdly, we often observed differences between men and women in associations between nut or peanut butter intake and cancer risk. We described several potential explanations for these differences previously, and it would be interesting to further investigate these sex differences. Firstly, we recommend to confirm the sex difference in other prospective studies by investigating sex-specific associations between nut intake and cancer risk. In addition, we suggest to study the hormonal, genetic, and molecular mechanisms that may underlie the observed differences.

We also recommend future studies to include multiple measurements of the exposure variables to be able to account for changes in diet and lifestyle over time. Moreover, it would be interesting to investigate subtypes of tree nuts and the influence of the processing of nuts, because differences in nutrient composition may result in differential effects on the associations with cancer risk.

Additionally, we suggest to examine the effects of nut and peanut butter consumption during childhood and (early) adolescence on the lifetime risk of cancer. Several studies have demonstrated that environmental and dietary exposures during preadolescence, adolescence, and early adulthood are more important in breast cancer development than exposures later in life, because breast tissue is most vulnerable to breast cancer development during those critical live periods (26, 27). It is therefore interesting to investigate whether preadolescent or (early) adolescent nut and peanut butter intake are associated with a reduced cancer risk.

Lastly, although slightly out of the scope of this thesis, the inverse associations between nut intake and cancer-related mortality could be the result of a lower risk of cancer, which we indeed observed for several cancer (sub)types in our study, but could also relate to improved survivorship after cancer diagnosis. Therefore, we recommend to investigate the effect of nut consumption on cancer prognosis and survival as well.

Implications – a broader perspective on the effects of nut consumption

Beneficial effects of nut consumption

This thesis provides evidence that increased nut consumption might be associated with a reduced risk of certain cancer (sub)types in men and women, while the effects of peanut butter intake are inconsistent. Based on literature, the beneficial effects of nut intake appear to extend beyond their potential cancer preventive effects and might also include reduced mortality and a lower risk of several other diseases.

Increasing nut intake has been found to reduce all-cause mortality and mortality related to cancer, cardiovascular diseases, stroke, coronary heart disease, respiratory diseases, neurodegenerative diseases, infectious diseases, and kidney diseases (9, 35-39). In addition, nut consumption might decrease the risk of obesity, have beneficial effects on post-prandial glycemia, insulin sensitivity, and on certain markers of lipid profile, including cholesterol and low-density lipoprotein concentrations, reduce the risk of hypertension, lower the systolic blood pressure, and possibly reduce the risk of type 2 diabetes (2, 38, 39). Also, an inverse relation between nut intake and gallstone disease has been observed (2, 39). Moreover, nut consumption has been associated with a reduced risk of depression, mild cognitive disorders, Alzheimer's disease, and impairment of physical functioning at older age, although the evidence for these associations is still very limited (38, 40).

Besides the beneficial health effects of nut consumption, dietary transition towards greater consumption of nuts as source of proteins might also beneficially impact the environment. The production and consumption of nuts has a much smaller impact on the greenhouse gas emission, land use, and nutrient pollution of the environment (both acidification and eutrophication) compared to, for example, dairy, fish, chicken, and unprocessed and processed red meat (41). The environmental impact varies because of differences among foods within a food group, the production method, and production location. However, those sources of variation have been found to have a smaller environmental impact than the food choice itself (41). Accordingly, food transition by incorporating nuts in healthier diets may not only contribute to an improved health but also to a more sustainable environment.

Possible risks of nut consumption

Weight gain and obesity

As described before, nuts are not only nutrient dense, but also very energy dense, because of their high fat content (1, 2). Therefore, concerns have been raised whether high nut consumption may result in weight gain and an increased risk of becoming obese in the

long term. Nevertheless, the opposite appears to be true: in a systematic review of six prospective cohort studies, long-term nut intake was inversely associated with weight gain and the risk of becoming overweight or obese (42). In the NLCS, we also observed that participants with a higher nut intake had, on average, a similar (men) or lower BMI (women) than nonconsumers at baseline (9, 12, 43).

Several mechanisms linking nut consumption to reduced weight gain and a lower risk of becoming overweight or obese have been hypothesized. These include that nuts might stimulate the secretion of satiety gut hormones, increase thermogenesis and resting energy expenditure, increase satiety signals induced by delayed gastric emptying, enhance fat oxidation, decrease the production of proinflammatory mediators, and improve insulin sensitivity (42).

In conclusion, at this moment, there is no prospective evidence that increased nut consumption results in weight gain. On the contrary, nut consumption has been associated with less weight gain and a reduced risk of becoming overweight or obese in several prospective studies (42).

Allergy

Tree nut and peanut allergies are common causes of life-threatening allergic reactions and have been associated with fatal episodes of anaphylaxis (44, 45). These allergies are caused by an immunological reaction to certain nut allergens that results in non-tolerance to these food items, mediated by immunoglobulin E (IgE) antibodies (45). Tree nut and peanut allergies mainly affect young children, of which less than 10% eventually becomes tolerant to tree nuts and less than 20% to peanuts (46). The prevalence rate of IgE-mediated tree nut allergy that was confirmed by food challenge tests or recent IgE testing (<2 years; by skin prick test or specific IgE) was less than 2% in children aged 0-18 years (44), and the prevalence of peanut allergy was approximately 2.5% in US pediatric populations (47). The risk of tree nut and peanut allergies is thus not very high. Nevertheless, given that tree nut and peanut allergies can be life-threatening, it is important to take this risk of nut consumption into account.

If an allergy is established, it is necessary to prevent subsequent allergic episodes that often tend to be clinically worse. At this moment, no cures for food allergies are available, and therefore avoidance of nuts and management of symptoms are the only options (45). This can be achieved by educating patients and their families to avoid all nut types and hidden nut products in processed foods, and to prevent cross contact of foods with the allergens during food preparation and serving (45). Also, patients and their relatives should be trained to recognize allergic reactions and act quickly to treat anaphylactic episodes, e.g. with antihistamines for non-severe allergic reactions and epinephrine for severe systemic allergic reactions (45).

Aflatoxin exposure

Another potential risk of tree nuts and peanut consumption is potential exposure to

aflatoxins, although this is relatively uncommon in Western countries. Aflatoxins are a highly toxic and carcinogenic group of secondary metabolites that are mainly produced by the mycotoxigenic fungi *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (48-50), which can typically be found in (sub)tropical regions with high temperatures and high relative humidity (49). These fungal species may colonize various staple foods including wheat, maize, spices, milk, tree nuts, and peanuts (49). Exposure to aflatoxins increases the risk of acute and chronic aflatoxicosis and hepatocellular carcinoma. Symptoms of acute aflatoxicosis, resulting from high dose exposures to aflatoxins, include acute hepatitis, edema, hemorrhagic necrosis of the liver, profound lethargy, and death (49, 50). Chronic aflatoxicosis is characterized by an increased risk of immune suppression, growth retardation, and eventually hepatocellular cancer, which is an important cause of cancer-related mortality worldwide (48-50).

The growth of aflatoxin-producing fungi and the level of aflatoxin contamination are affected by several factors during every step of food production, from pre-harvest to storage (49). These factors include, amongst others, the climate, soil type, humidity, daily temperatures, insect activity, stress to crops due to drought, and inadequate drying before storage (49). Because of its severe toxic effects and hepatocarcinogenic potential, aflatoxin exposure is highly regulated in many countries worldwide (51), e.g. strict regulations prohibit the sale of crops contaminated with aflatoxins (49). However, aflatoxin exposure is still one of the major risk factors for hepatocellular carcinoma, especially in developing countries in (sub) tropical regions, given their beneficial climate for fungal contamination and their reliance on aflatoxin-susceptible staple foods due to food insufficiency and lack of food diversity (49).

Balancing the risks and benefits of nut consumption

When balancing the beneficial effects and risks, we conclude that the possible benefits of increasing nut consumption as part of a healthy lifestyle outweigh its potential disadvantages. Nuts have been associated with a reduced risk of several cancer (sub)types, many other diseases, and with reduced overall and cause-specific mortality. For peanut butter, the evidence is limited and inconsistent, and it should thus not be regarded as a substitute for nuts. In addition, nuts may contribute to more sustainable nutrition with less impact on the environment. Increasing nut consumption has not been associated with weight gain or an increased risk of becoming overweight or obese, but rather with a reduced risk of these health issues. Moreover, although aflatoxin exposure might be a substantial hazard in some countries, this is hardly the case in the Netherlands because of strict regulations and quality control, and because the Dutch climate is suboptimal for mycotoxins. The risk of nut allergy is relatively low, but remains an important concern, especially among children. Obviously, people who are allergic to tree nuts or peanuts should avoid them.

Concluding remarks on nut consumption and cancer risk

The general aim of this thesis was to investigate the associations between total nut, tree nut, peanut, and peanut butter and the risk of several cancer (sub)types. Our results confirm

that nuts show promising potential in the prevention of cancer, although the effects were not very strong, differed between men and women, and were not seen for all cancer (sub) types. Moreover, the beneficial effects of nut intake appear to extend beyond their potential cancer preventive effects and might also include reduced mortality and a lower risk of several other diseases. For peanut butter, the evidence concerning cancer risk was less consistent. Therefore, peanut butter cannot be considered as a substitute for nuts. In conclusion, nuts might represent a relatively cheap option that might contribute to the prevention of the worldwide public health problem of cancer, and can be readily incorporated into healthy diets.

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Addendum

Summary

Interest in the health effects of nut consumption has been increasing rapidly since nut intake was found to protect against coronary heart disease in 1992. Apart from its effect on cardiovascular diseases, nut intake has recently also been associated with a reduced risk of other non-communicable diseases and mortality, including cancer-related mortality. The effects on cancer-related mortality were substantial, and therefore nuts might offer promising potential for cancer prevention. Primary prevention of cancer is very important, because cancer incidence remains high and because of the poor prognosis of some cancer types. However, prospective evidence on the relation between nut consumption and cancer risk is limited.

For this reason, the primary aim of this thesis was to investigate the associations between nut and peanut butter intake and the risk of cancer in men and women in the prospective Netherlands Cohort Study on diet and cancer (NLCS). More specifically, we studied the associations of total nut, tree nut, peanut, and peanut butter intake with the risk of esophageal, gastric, colorectal, pancreatic, lung, breast, endometrial, ovarian, prostate, and total cancer in men and women. As secondary aim, we investigated the associations between nut and peanut butter intake and the risk of colorectal tumors harboring *APC*, *KRAS*, or *BRAF* mutations, p53 overexpression, or microsatellite instability, to account for molecular heterogeneity and to provide new insights in the possible involvement of these genes in the associations between nut intake and colorectal cancer development.

The NLCS was initiated in September 1986 and included 120,852 men and women aged 55-69 years, from 204 Dutch municipal population registries. At baseline, participants completed a self-administered 11-page baseline questionnaire on lifestyle, dietary intakes, and other potential cancer risk factors. This baseline questionnaire included a validated 150-item food frequency questionnaire (FFQ) that focused on habitual food consumption during the year preceding baseline. A case-cohort approach was applied, in which cases were derived from the entire cohort, whereas the person-years at risk for the total cohort were estimated using a subcohort. This subcohort consisted of 5000 participants who were randomly sampled from the entire cohort at baseline, and was followed-up biennially for information on vital status. Cancer cases in the total cohort were identified through annual record linkage with the Netherlands Cancer Registry and the Netherlands Pathology Registry (PALGA). In this thesis, we used data from the follow-up period September 1986-December 2006 (20.3 years of follow-up). For the analysis of the molecular subtypes of colorectal cancer, the follow-up period September 1986-December 1993 (and excluding the first 2.3 years) was used because tumor material has been collected for colorectal tumors that occurred during this period for an earlier project.

In Chapter 2, we observed that increased nut intake was associated with a statistically significant decreased risk of esophageal squamous cell carcinoma and gastric non-cardia adenocarcinoma. A nonsignificant positive association was seen for the risk of esophageal adenocarcinoma, and no clear association was found for gastric cardia adenocarcinoma.

For peanut butter consumption, nonsignificant positive associations were found for the esophageal cancer subtypes, but not for the gastric cancer subtypes.

In Chapter 3, we observed significant nonlinear inverse associations with rectal cancer for total nut, peanut, and peanut butter intake in women in restricted cubic spline analyses, and borderline significant nonlinear inverse associations for total nut and peanut intake in men. No significant associations were observed for other anatomical CRC subtypes. Regarding the molecular CRC subtypes after 7.3 years of follow-up, peanut butter intake was significantly associated with an increased risk of colorectal tumors that did not develop through the serrated neoplasia pathway in men.

Nut intake was non-significantly inversely associated with microscopically confirmed pancreatic cancer risk in men, and a significantly reduced risk was observed for peanut butter intake (Chapter 4). In women, no significant associations with microscopically confirmed pancreatic cancer were seen for nut or peanut butter intake. The observed associations were weaker when looking at total pancreatic cancer risk.

In Chapter 5, total nut, tree nut, and peanut intake were significantly related to a reduced risk of small cell lung carcinoma in men, after controlling for detailed smoking habits. No significant associations were found in men for the other lung cancer subtypes or total lung cancer, in women, or for peanut butter intake.

A significant inverse association was found between nut intake and the risk of estrogen receptor (ER)-negative postmenopausal breast cancer (Chapter 6). There were no significant inverse associations with ER-positive or total breast cancer risk. There was no variation between progesterone receptor (PR) subtypes, although the ER-negative PR-negative subtype was also significantly inversely associated with nut intake. No significant associations were found between peanut butter intake and breast cancer risk or its ER/PR-subtypes.

In Chapter 7, no significant association between the intake of total nuts, tree nuts, peanuts, and peanut butter and the risk of endometrial or ovarian cancer were observed. Significant interactions were seen in the endometrial cancer analyses, in particular an interesting interaction between total nut intake and cigarette smoking status, in which increasing nut consumption appeared to attenuate the inverse association between cigarette smoking and endometrial cancer risk.

No significant associations were seen between total nut, tree nut, and peanut intake and the risk of total, advanced, and non-advanced prostate cancer (Chapter 8). Peanut butter consumption was significantly positively associated with the risk of non-advanced prostate cancer.

Finally, in Chapter 9, total nut, tree nut, peanut, and peanut butter intake were not associated with the risk of total cancer in men or women. Moreover, no associations were found with smoking-(un)related and alcohol-(un)related cancers in both sexes.

This thesis concludes with a summary of the main findings, overall interpretation, a discussion of methodological considerations, implications, and recommendations for future research in Chapter 10. Overall, our results indicate that nut consumption shows promising potential in the prevention of several cancer types, including esophageal squamous cell carcinoma, gastric non-cardia adenocarcinoma, rectal cancer, small cell lung carcinoma (in men), and ER-negative and ER-negative PR-negative postmenopausal breast cancer (in women). However, the protective effects were not very strong and differed between men and women. For peanut butter intake, the associations with cancer risk were less consistent. Nuts might thus represent a relatively cheap option that might contribute to the prevention of the worldwide public health problem of cancer and to the prevention of other diseases. Nuts can be readily incorporated into healthy diets, although it is important to be aware of potential tree nut and peanut allergies.

Nederlandse samenvatting

De interesse in de gezondheidseffecten van nootconsumptie is snel toegenomen sinds aangetoond werd dat nootconsumptie bescherming biedt tegen coronaire hartziekten in een studie in 1992. Naast dit effect op hart- en vaatziekten is de inname van noten recentelijk ook geassocieerd met een verlaagd risico op andere niet-overdraagbare ziekten en sterfte, inclusief sterfte door kanker. De effecten op sterfte door kanker waren aanzienlijk en daarom bieden noten mogelijk een veelbelovende optie in kankerpreventie. Primaire preventie van kanker is erg belangrijk, vanwege de hoge kankerincidentie en de slechte prognose van sommige kankertypes. Prospectief bewijs voor een relatie tussen nootconsumptie en kankerrisico is echter beperkt.

Het hoofddoel van dit proefschrift was om de associaties tussen de inname van noten en pindakaas en het risico op kanker bij mannen en vrouwen te onderzoeken in de prospectieve Nederlandse Cohort Studie naar voeding en kanker (NLCS). We hebben de associaties tussen de inname van noten, pinda's en pindakaas en het risico op slokdarm-, maag-, colorectaal-, alvleesklier-, long-, borst-, endometrium-, ovarium-, prostaat- en totale kanker onderzocht in mannen en vrouwen. Als secundair doel onderzochten we de associaties tussen de inname van noten en pindakaas en het risico op colorectale tumoren met *APC*-, *KRAS*- of *BRAF*-mutaties, overexpressie van p53 of microsatelliet instabiliteit, om rekening te houden met moleculaire heterogeniteit en om nieuwe inzichten te verkrijgen in de mogelijke betrokkenheid van deze genen in de associaties tussen de inname van noten en de ontwikkeling van colorectaalkanker.

De NLCS is gestart in september 1986 en bestaat uit 120.852 mannen en vrouwen in de leeftijd 55-69 jaar, afkomstig uit 204 Nederlandse gemeentelijke bevolkingsregisters. Bij aanvang van de studie vulden alle deelnemers een vragenlijst in over leefstijl, voedingsgewoontes en andere potentiële risicofactoren voor kanker. De vragenlijst bestond uit 11 bladzijdes en omvatte ook een gevalideerde voedingsvragenlijst over 150 voedingsitems, gericht op de gebruikelijke voedselconsumptie in het jaar voorafgaand aan de start van de studie. Er werd een case-cohortbenadering toegepast, waarbij kankerpatiënten afkomstig waren uit het gehele cohort, terwijl het aantal persoonsjaren at risk voor het totale cohort werd geschat met behulp van een subcohort. Dit subcohort bestond uit 5000 deelnemers die bij aanvang van de studie willekeurig geselecteerd werden uit het totale cohort en die tweejaarlijks werden opgevolgd voor informatie over hun vitale status. Kankerpatiënten in het totale cohort werden geïdentificeerd door middel van een jaarlijkse koppeling met de Nederlandse Kankerregistratie en het Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief (PALGA). In dit proefschrift hebben we gegevens gebruikt uit de follow-up periode september 1986-december 2006, oftewel een periode van 20,3 jaren. Voor de analyse van de moleculaire subtypes van colorectaalkanker is de follow-up periode september 1986-december 1993 (exclusief de eerste 2,3 jaren) gebruikt omdat materiaal van colorectale tumoren alleen verzameld is gedurende deze periode voor een eerder project.

In Hoofdstuk 2 vonden we dat een hogere nootinname geassocieerd was met een statistisch significant verlaagd risico op plaveiselcelcarcinoom van de slokdarm en non-cardia adenocarcinoom van de maag. Een niet-significante positieve associatie werd gezien voor het risico op adenocarcinoom van de slokdarm, en er werd geen duidelijke associatie gevonden voor cardia adenocarcinoom van de maag. Voor de consumptie van pindakaas werden niet-significante positieve associaties gevonden voor de subtypes van slokdarmkanker, maar niet voor de subtypes van maagkanker.

In Hoofdstuk 3 hebben we significante niet-lineaire inverse associaties met rectumkanker waargenomen in vrouwen voor totale nootinname en voor de inname van noten, pinda's en pindakaas apart in zogeheten 'restricted cubic spline analyses' (waarin lineariteit niet verondersteld wordt) en net significante niet-lineaire inverse associaties voor de totale inname van noten en pinda's in mannen. Er werden geen significante associaties waargenomen voor de andere anatomische subtypes van colorectaalkanker. In de analyses van de moleculaire colorectaalkanker-subtypen was een hogere pindakaasconsumptie significant geassocieerd met een verhoogd risico op colorectale tumoren die zich niet ontwikkelden via de serrated neoplasia pathway in mannen na 7,3 jaren follow-up.

Een hogere nootconsumptie was niet-significant geassocieerd met een lager risico op microscopisch bevestigde alvleesklierkanker bij mannen en er werd een significant verlaagd risico waargenomen voor de inname van pindakaas (Hoofdstuk 4). Bij vrouwen werden geen significante associaties gezien met microscopisch bevestigde alvleesklierkanker voor de inname van noten of pindakaas. De waargenomen associaties waren zwakker wanneer gekeken werd naar het risico op totale alvleesklierkanker.

In Hoofdstuk 5 was een hogere totale nootinname en een hogere inname van noten en pinda's afzonderlijk significant gerelateerd aan een verlaagd risico op kleincellig longcarcinoom bij mannen, zelfs nadat we uitgebreid gecorrigeerd hadden voor rookgewoonten. Er werden geen significante associaties gevonden bij mannen voor de andere subtypes van longkanker of totale longkanker, bij vrouwen of voor pindakaasconsumptie.

Een significant verband is gevonden tussen een hogere nootconsumptie en een lager risico op estrogeenreceptor (ER)-negatieve postmenopauzale borstkanker (Hoofdstuk 6). Er waren geen significante inverse associaties met het risico op ER-positieve of totale borstkanker. Er was geen variatie tussen de progesteronreceptor (PR) subtypes, hoewel het ER-negatieve PR-negatieve subtype ook significant invers geassocieerd was met nootconsumptie. Er werden geen significante associaties gevonden tussen de inname van pindakaas en het risico op borstkanker of de ER/PR-subtypes.

In Hoofdstuk 7 werd geen significant verband waargenomen tussen de inname van totale noten, noten, pinda's en pindakaas afzonderlijk en het risico op endometrium- of ovariumkanker. Significante interacties werden gezien in de endometriumkankeranalyses, en dan met name een interessante interactie tussen totale nootconsumptie en het roken van sigaretten, waarbij een hogere nootconsumptie de inverse associatie tussen het roken

van sigaretten en het risico op endometriumkanker lijkt te verzwakken.

Er werden geen significante associaties gezien tussen totale nootinname of de inname van noten en pinda's individueel en het risico op totale, gevorderde en niet-gevorderde prostaatkanker (Hoofdstuk 8). Een hogere consumptie van pindakaas was significant geassocieerd met een verhoogd risico op niet-gevorderde prostaatkanker.

Ten slotte waren in Hoofdstuk 9 totale nootconsumptie en de consumptie van noten, pinda's en pindakaas niet geassocieerd met het risico op totaal kanker in mannen of vrouwen. Bovendien werden bij beide geslachten geen associaties gevonden tussen noot- en pindakaasconsumptie en rook- en alcohol-(on)gerelateerde kankers wanneer we deze kankertypes als aparte groepen analyseerden.

Dit proefschrift sluit af met een samenvatting van de belangrijkste bevindingen, algemene interpretatie, bespreking van methodologische overwegingen, implicaties en aanbevelingen voor toekomstig onderzoek in Hoofdstuk 10. Over het algemeen laten onze resultaten zien dat nootconsumptie een veelbelovende optie kan zijn in de preventie van een aantal kankertypes, waaronder plaveiselcelcarcinoom van de slokdarm, non-cardia adenocarcinoom van de maag, rectumkanker, kleincellig longcarcinoom (in mannen) en ER-negatieve en ER-negatieve/PR-negatieve postmenopauzale borstkanker (in vrouwen). De beschermende effecten waren echter niet erg sterk en verschilden tussen mannen en vrouwen. Voor pindakaasconsumptie waren de associaties met het risico op kanker in het algemeen minder eenduidig. Noten zouden dus een relatief goedkope optie kunnen zijn die zou kunnen bijdragen aan de preventie van het wereldwijde gezondheidsprobleem van kanker, en mogelijk ook aan de preventie van andere ziekten. Noten kunnen gemakkelijk worden opgenomen in een gezond dieet, hoewel het daarbij belangrijk is rekening te houden met mogelijke noten- en pinda-allergieën.

Impact

Valorization, by definition, is the process of creating value and impact from knowledge, by making knowledge suitable and/or available for economic and societal utilization, and by translating knowledge into new products, services, processes, or business (1). By law, valorization constitutes the third core task of Dutch universities, in addition to research and education. In this addendum, the societal and economic relevance of the results presented in this thesis will be discussed.

From scientific to societal and economic value

Cancer is a huge public health problem worldwide, with approximately 18.1 million incident cancer cases and 9.6 million cancer deaths globally in 2018 (2). The global burden of cancer is expected to rise to 29.5 million new cancer cases and 16.4 million cancer-related deaths by 2040 due to the growth and ageing of the population (3). Because of the high number of cancer cases and deaths and because of the expected rise in these numbers, we cannot treat our way out of the cancer problem (4). Therefore, primary prevention of cancer is of great importance. It has been estimated that approximately 42-50% of all cancers can potentially be prevented by avoiding modifiable risk factors (4, 5). Moreover, in 2010, about 10% of all cancer diagnoses in the Netherlands could be attributed to a suboptimal diet (6). Consequently, society could benefit from nutritional research by incorporating this scientific knowledge into dietary guidelines. In the view of the above, the results presented in this thesis may have an impact in several ways.

In this thesis, we tested a novel hypothesis concerning the potential inverse relation between nut and peanut butter intake and cancer risk. Our study results showed that nut intake is significantly inversely associated with several cancer (sub)types, including esophageal squamous cell carcinoma, gastric non-cardia adenocarcinoma, rectal cancer, small cell lung carcinoma (in men), and ER-negative and ER-negative/PR-negative postmenopausal breast cancer (in women). Most of these cancer types have a poor prognosis, which makes preventive strategies even more important. As described in the Discussion (Chapter 10), the literature on nut and peanut butter consumption in relation to cancer risk is still rather limited. However, the beneficial effects of nut consumption seem to extend beyond their potential cancer-preventive properties and the evidence for an inverse relation between nut consumption and cardiovascular diseases is much stronger. Thus, together with previous and future research findings, our study results could thus jointly result in e.g. dietary recommendations and guidelines.

An example of such dietary guidelines is the World Cancer Research Fund (WCRF) Continuous Update Project (CUP), in which literature on diet and other lifestyle factors in relation to cancer risk and mortality is systematically reviewed. In the third expert report, nuts were mainly included in the category of foods containing dietary fiber (7). In future expert reports of the WCRF CUP, our study results might support recommendations for nut intake as food item. The recommendations in the WCRF CUP enable everyone, from members of the public

to policy makers, to have access to the most recent scientific knowledge on how to prevent cancer development.

Moreover, the Health Council of the Netherlands advises the Dutch authorities on health recommendations. In the Advisory report Dutch dietary guidelines 2015 it was recommended to eat at least 15 grams of unsalted nuts daily (8). This recommendation was based on studies that showed that nut consumption reduces LDL cholesterol and is associated with a reduced risk of coronary heart disease. In future health recommendations of the Health Council, the beneficial effects on cancer risk may be incorporated as well. Based on these dietary recommendations of the Health Council of the Netherlands, the Netherlands Nutrition Center (Voedingscentrum) developed and released their latest food pyramid, called 'the Wheel of Five' or 'De Schijf van Vijf' in Dutch, in 2020 (9). One of the seven recommendations in the Wheel of Five is to eat a handful (25 grams) of unsalted nuts daily. This food pyramid is an easy understandable tool for consumers to improve their eating habits.

Incorporating nuts as part of a healthy diet and lifestyle will hopefully eventually lead to a decrease in morbidity and mortality related to cancer. Decreasing the cancer morbidity and mortality will also lead to a lower economic burden of cancer related to health care costs and productivity losses. Because the beneficial effects of nut intake go beyond cancer and also include, amongst others cardiovascular, respiratory, neurodegenerative, infectious, and kidney diseases (10-15), the societal and economic impact of increasing nut consumption as part of a healthy lifestyle may be even more far-reaching.

Knowledge transfer

The scientific knowledge obtained in this research project has been shared with other experts in this field through publication of our study results in internationally renowned scientific journals. Most articles have been published open access in order to disseminate our results more rapidly and widely. Of the remaining articles that were not published open access, the postprints have been deposited in the open access UM repository PURE. In addition, our articles published until 2019 have been included in two recent meta-analyses (16, 17), which likely reach an even larger audience than the individual reports. Moreover, the results of this research project have been displayed and presented at the Dutch Epidemiological Conference (WEON) in Bilthoven in 2018 and in Groningen in 2019. The audience of this conference mainly consists of epidemiologists. In addition, our study results were also presented at the Society for Social Medicine and Population Health & International Epidemiology Association Joint Scientific Meeting in Cork, Ireland, in 2019. Unfortunately, presentations at congresses in 2020 were cancelled due to the coronavirus pandemic.

Conclusion

In this thesis, we investigated the associations between nut and peanut butter intake and the risk of several cancer (sub)types. The results could be used by researchers, policy

makers, and the public to decrease the risk of several chronic conditions, including several cancer (sub)types. This might be achieved by incorporating the knowledge obtained in this thesis jointly with previous and future studies into dietary guidelines. Eventually, this may also result in lower health care costs and productivity losses. In addition, our results provide new leads for future research that may also contribute to new insights in the field of cancer etiology. In conclusion, our results indicate that nut consumption as part of a healthy diet and lifestyle is a promising strategy in the prevention of several cancer (sub)types.

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Curriculum Vitae

Lisette Nieuwenhuis was born on December 7th 1993 in Enschede, the Netherlands. After graduating from secondary school with honours at Bonhoeffer College in Enschede in 2011, she studied Biomedical Sciences at the Radboud University in Nijmegen. She obtained her bachelor's degree in 2013 (bene meritum). During her master's education she specialized in the fields Health Technology Assessment and Epidemiology. Lisette wrote her master's thesis "Cost-effectiveness of longer-term versus shorter-term provision of antibiotics in patients with persistent symptoms attributed to Lyme disease" at the Departments of Health Evidence and Internal Medicine of the Radboud University Medical Center. After obtaining her master's degree in 2016 (bene meritum), she started working as a PhD student at the Department of Epidemiology of the Maastricht University (CAPHRI – Care and Publich Health Research Institute). Under the supervision of Prof. dr. ir. Piet A. van den Brandt and dr. Colinda C.J.M. Simons, she studied the associations between nut and peanut butter consumption and the risk of cancer in the Netherlands Cohort Study on diet and cancer. The scientific results of this project, which are presented in this thesis, have been published in international peer-reviewed journals and have been presented at (inter)national congresses. Currently, Lisette is working as a lecturer and researcher at the Department of Health Technology and Services Research of the University of Twente in Enschede.

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